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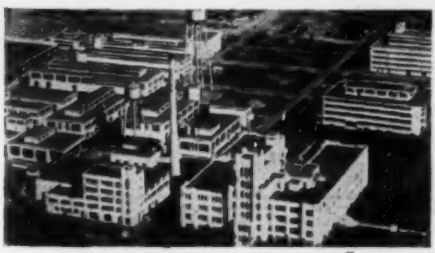

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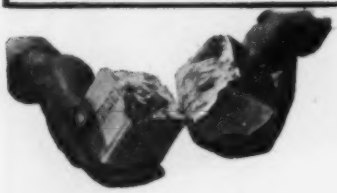

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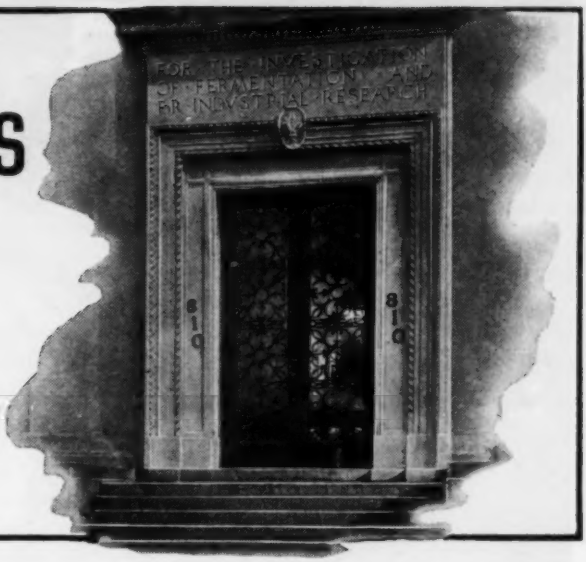

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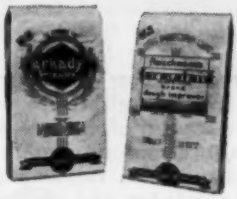

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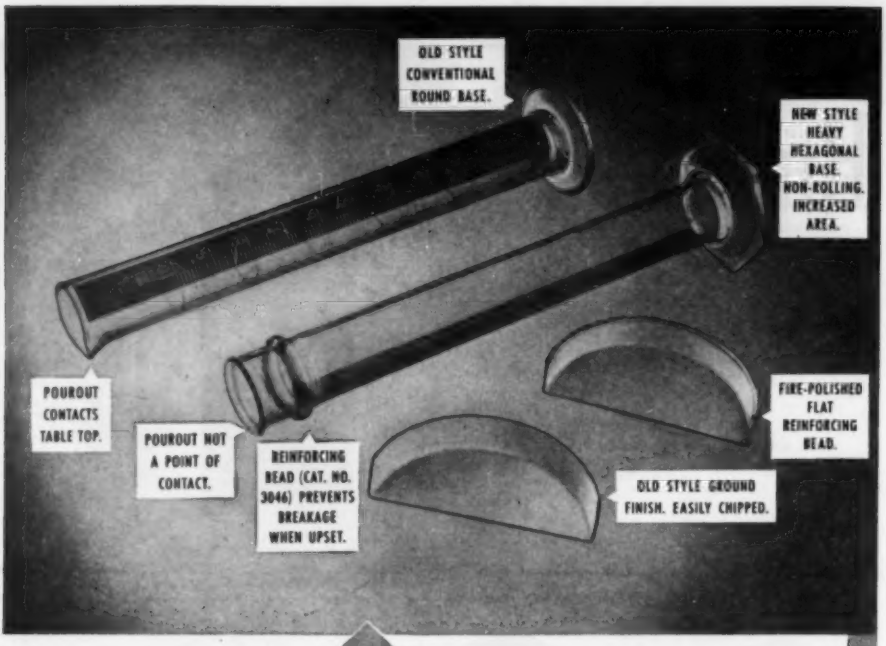
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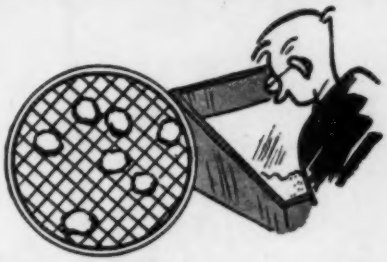

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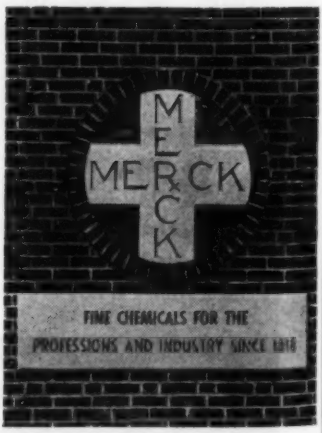

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CEREAL CHEMISTRY

VOL. XXII NOVEMBER, 1945 No. 6

WHEAT STARCH PROPERTIES IN RELATION TO GRAIN MATURITY

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(Received for publication June 30, 1945)

No work has been reported on changes in composition and properties of wheat starch granules at different stages of maturity, and very little on the size and form of the granules. Brenchley (1909), Brenchley and Hall (1909), Thatcher (1913, 1915), Percival (1921), and Belval (1924) followed histologically or analytically the development of the wheat grain and the deposition of starch within the endosperm, but they made no specific studies of starch characteristics during maturation of the grain.

The present study was undertaken to obtain information on the rapidity of starch deposition and to determine possible changes in size, form, chemical composition, and viscosity of wheat starch granules at different stages of maturity of the grain. Experiments were extended to cover the period of storage immediately following harvest of the grain, when, as a consequence of the drying of the wheat kernels, changes might occur.

Experimental

Wheat Samples. Thorne, Iowin, and Pilot 13 wheats, which represent soft red winter, hard red winter, and hard red spring wheat respectively, were grown near Peoria, Illinois, in the 1942 crop year.

Time of pollination of wheat can be closely determined by observation of anthesis, which occurs when the stigma becomes receptive. Percival (1921) found that only about 20 minutes are required for the opening and closing of the wheat flower. During this time dehiscence and extrusion of the anthers occur, with consequent shedding of the

[1] A considerable portion of this work was presented in partial fulfillment of requirements for the M.S. degree at Fort Hays Kansas State College, Hays, Kansas, July 1943, by Claude W. Bice.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

pollen. Fertilization takes place in from 1 to 2 days after pollination (Brenchley, 1909; Percival, 1921). In the present study, heads were marked by colored string tied around the stem at the time of pollination, i.e., when anthesis was observed.

The wheats were harvested at intervals, varying from 2 to 7 days, throughout the growing season. Each sample consisted of 300 heads, hand-harvested, picked at random, which were collected in the early morning and were held in the refrigerator at about 5°C until they were used. Sterile and dwarf spikelets were removed from the spike before the caryopses, or kernels, were picked or threshed from the heads. Three samples of each wheat were studied during a 5½-week storage period immediately after harvest.

In the earlier stages 3 to 4 hours were often required to remove by hand all the kernels constituting one sample. Only a few heads were taken from the refrigerator at a time; the kernels were removed and dropped at once into iced, distilled water to retard possible enzymic changes. Near the season's end, as the wheat began to ripen, it was possible to thresh the grain rapidly from the heads, first, by rubbing the latter between the palms of the hands or, later, by machine.

Preparation of Starch. Starch was prepared on the same day of harvesting, separation being completed within 10 hours after the wheat heads were picked. Grain collected from 2 to 15 days, inclusive, after pollination was ground through a Russwin No. 2 handmill into ice water, and the starch was removed by working the ground material on No. 13 standard bolting silk. Repeated washing and centrifuging served to separate the starch from most of the other material that passed through the silk; the latter material, when centrifuged, formed a layer above the starch and was scraped away. In the earlier stages of maturity, up to about 12 days after pollination, separation of starch was usually so poor that the crude starch had to be cleaned by sedimentation from a relatively long column of distilled water.

Wheat collected 20 days or more after pollination was, in general, processed without use of sulfur dioxide by a standard laboratory method (MacMasters and Hilbert, 1944, method 1). The following slight modifications, however, were made: (a) Grain harvested from 20 to 32 days after pollination was still sufficiently moist to permit immediate grinding, and steeping was therefore omitted. Samples harvested more than 32 days after pollination were steeped for 24 hours in distilled water at 5°C. (b) The starch that was isolated was dried at room temperature with occasional stirring.

Analytical and Physical Methods. Moisture, nitrogen, phosphorus, and ash were determined as described by MacMasters and Hilbert (1944a).

Viscosity determinations were made in a Stormer viscometer (MacMasters and Hilbert, 1944). The procedure employed is rapid and is useful for comparative purposes in following possible gross differences between starches. Incomplete data are obtained by this procedure, since a full characterization of each starch would require detailed curves showing viscosity changes with concentration, temperature, and varied rates of shear. The size of available starch samples did not permit the collection of such information, particularly for starch obtained at the earlier stages of maturity, where the results would be of most interest.

Transmittancy curves of starches during pasting were obtained by Morgan's (1940) method with slight modifications in the apparatus made at this laboratory by S. A. Karjala (unpublished).

The method described by Bates, French, and Rundle (1943) was used for determining iodine sorption by the starches. Before analysis, the starches were extracted in a Soxhlet or Butt type extractor with 85% methyl alcohol for a period of time sufficient to remove most of the fatty acids. This extraction method has been found to leave about 0.05% fatty material in the starch. Results are reported as milligrams iodine sorbed per gram of starch. These values are calculated to show tentative percent amylose present, on the assumption that pure amylose sorbs 200 mg iodine per gram (unpublished data obtained in this laboratory by I. A. Wolff and R. L. Whistler). It is, however, fully recognized that amyloses from different sources may have different sorption values, and that calculations based on a "standard amylose" value are purely empirical.

Granule sizes were determined by means of a microscope equipped with a calibrated filar micrometer eyepiece. All samples were examined with a polarizing microscope to determine whether the granules were birefringent. At the same time, shape and general appearance of the granules were noted.

Data Obtained. Data were obtained on weight of spikes, of normal grain, and of starch recovered from the three wheats at several stages of maturity. These and related harvesting data are summarized in Table I. Harvesting and separation of the grain at early stages of maturity were so slow and tedious that determination of moisture in the spikes and whole grain was not made. Values given in the column headed "percent grain from spikes" were calculated as

$$\frac{\text{Wt. normal grain from 300 undried spikes}}{\text{Wt. undried 300 spikes after removal of dwarf spikelets}} \times 100.$$

The percent of grain obtained from spikes increased as the grain matured. This was probably caused by an increase in weight of grain

TABLE I

DATA ON HARVEST OF WHEAT AND ON YIELD OF STARCH AT DIFFERENT STAGES OF GRAIN MATURITY [1]

Wheat, variety and class	Stage	Days after pollination	Wt. 300 spikes after removal of dwarf spikelets [2]	Normal grain from 300 spikes		Starch removed from 300 spikes	
				Weight	Proportion of spike	Weight	Proportion of grain
			g	g	%	g	%
Thorne, soft red winter	Fertilization	2	160	29	18	1.3	4.5
	Early milk	8	282	158	56	13.3	8.4
	Milk	10	259	165	64	20.8	12.6
	Late milk	12	241	161	67	24.8	15.4
	Early dough	16	445	303	68	58.4	19.3
	Soft dough	20	375	266	71	75.6	28.4
	Hard dough	25	256	—	—	88.4	—
	Ripe	32	325	253	78	114.2	45.2
	Postharvest	41	342	277	81	107.0	38.6
	Postharvest	48	281	229	82	108.9	47.5
	Postharvest	55	298	244	82	109.2	44.8
Iowin, hard red winter	Milk	10	319	152	48	11.5	7.6
	Late milk	12	377	189	50	22.3	11.8
	Early dough	16	343	186	54	26.1	14.0
	Hard dough	24	320	226	70	72.5	32.1
	Ripe	32	257	200	78	82.1	41.0
	Postharvest	41	282	212	75	73.7	34.7
	Postharvest	48	241	180	75	84.0	46.6
	Postharvest	55	253	194	77	97.4	50.2
Pilot 13, hard red spring	Late milk	16	518	259	50	23.3	9.0
	Late milk	20	510	282	55	51.3	18.2
	Medium dough	24	635	336	53	66.7	19.9
	Medium dough	28	656	366	56	84.0	22.9
	Hard dough	32 [3]	517	284	55	63.5	22.4
	Ripe	38	367	256	70	93.5	36.5
	Postharvest	46	359	257	72	96.7	37.6
	Postharvest	53	302	208	69	85.5	41.0
	Postharvest	60	318	220	69	103.2	47.0

[1] Weight of spikes and grain given for undried material; weight of starch on air-dried basis (approx. 12% moisture).
[2] Based on actual weight of 30 spikes.
[3] 50% blight damage.

as well as by a decrease in moisture content of the entire spikes. After harvest the values fluctuated as a result, in part at least, of moisture differences.

Starch recovered from the sound grain is reported on the air-dried basis (air-dried starch contains about 12% moisture). Emphasis was placed on obtaining clean starch rather than maximum yield. In general, however, the yield, calculated as percent starch from grain, increased until maturity, after which it fluctuated slightly during the storage period, but tended to increase, perhaps because of greater

ease in separation of the gluten from the starch or because of loss of moisture from the grain during storage. A low percentage of small granules often indicated partial loss during processing of the fraction of starch comprising the small granules and was in many cases correlated with lower yield of starch. Values in the column giving "percent starch from grain" were calculated as:

$$\frac{\text{Wt. air-dried starch from normal grain from 300 undried spikes}}{\text{Wt. undried normal grain from 300 undried spikes}} \times 100.$$

The starch obtained from Thorne wheat 2 days after pollination came from tissues other than the endosperm. Microscopic examination of kernels sectioned at this stage showed that no endosperm tissue had as yet formed. All other starches, however, came mainly from the endosperm, although minor amounts from other kernel tissue could not be excluded.

TABLE II

DATA ON STARCH FROM WHEAT HARVESTED AT SUCCESSIVE STAGES OF MATURITY

Wheat	Days after pollination	Noncarbohydrate constituents (Moisture-free basis)			Granule size range in starch samples	Percentage of granules of starch samples falling into size groups		
		Nitrogen	Phosphorus	Ash		<11μ	11–22μ	>22μ
		%	%	%	μ	%	%	%
Thorne	2	—	—	—	1.1–7.8	100	0	0
	8	0.19	—	—	2.0–29	65	33	2
	10	0.16	—	—	1.8–30	63	33	4
	12	0.04	0.059	0.13	1.5–34	32	52	16
	16	0.04	0.059	0.16	1.8–40	45	40	15
	20	0.07	0.064	0.18	1.2–36	65	25	10
	25	0.04	0.067	0.18	1.2–40	77	17	6
	32	0.05	0.074	0.19	1.7–37	64	23	13
	41	0.04	0.066	0.14	1.7–41	58	21	21
	48	0.04	0.069	0.16	1.3–39	70	19	11
	55	0.04	0.069	0.15	2.2–37	60	24	16
Iowin	10	0.38	—	—	1.1–35	51	44	5
	12	0.39	—	—	1.1–33	60	35	5
	16	0.05	0.073	0.15	1.3–35	64	27	9
	24	0.04	0.068	0.14	1.6–36	78	12	10
	32	0.05	0.071	0.17	2.1–47	60	20	20
	41	0.04	0.067	0.12	2.2–45	35	44	21
	48	0.04	0.064	0.15	1.8–40	70	19	11
	55	0.05	0.067	0.15	2.2–45	72	16	12
Pilot 13	16	0.05	0.045	0.12	1.3–35	47	37	16
	20	0.04	0.056	0.13	1.5–37	24	58	18
	24	0.05	0.062	0.13	1.1–39	57	29	14
	28	0.04	0.064	0.12	0.9–37	82	12	6
	32	0.04	0.060	0.15	1.3–37	60	28	12
	38	0.04	0.064	0.14	1.8–39	53	31	16
	46	0.05	0.069	0.14	2.3–36	50	34	16
	53	0.04	0.065	0.12	2.2–36	43	33	24
	60	0.04	0.068	0.16	2.0–36	75	16	9

TABLE III

IODINE SORPTIVE CAPACITY OF DEFATTED STARCH
(Starch was defatted 48 hours with 85% methanol in a Soxhlet extractor)

Days after pollination	Iowin		Pilot 13		Thorne	
	Mg I sorbed per g starch	Calc'd.[1] per cent amylose	Mg I sorbed per g starch	Calc'd.[1] per cent amylose	Mg I sorbed per g starch	Calc'd.[1] per cent amylose
Days						
8	—	—	—	—	38.5	19
12	38.8	19	—	—	43.4	22
16	45.1	23	42.7	21	45.6	23
24	49.0	25	49.7	25	—	—
25	—	—	—	—	50.4	25
32	49.4	25	50.2	25	50.6	25
38	—	—	51.7	26	—	—
55	49.2	25	—	—	49.1	25
60	—	—	52.6	26	—	—
53	(Small granule fraction)		52.5	26	—	—

[1] Calculated on assumption that 1 g pure amylose sorbs 200 mg iodine.

Data on granule size, nitrogen, phosphorus, and ash content, and on iodine sorptive capacity are presented in Tables II and III. Phosphorus and ash were not determined in samples heavily contaminated with foreign material, as indicated by microscopic evidence. When so contaminated the starch sample always had a higher nitrogen content.

Discussion of Results

Starch was first deposited in the endosperm of Thorne wheat sometime between the second and the eighth day after pollination. On the second day after pollination an appreciable amount of starch was separated (see Table I). This, however, originated from pericarp tissue rather than from the endosperm and occurred as very small birefringent granules which stained blue with iodine (Figure 1). On the basis of Percival's (1921) observations, it would be expected that this starch was largely used up during the subsequent rapid growth of the grain. Endosperm starch was so abundant at the eighth day that it must be assumed to have first appeared some days earlier. When the weight of starch recovered was plotted against time after pollination, an almost linear relation was observed. Extrapolation of this curve permitted a tentative estimate of the initial appearance of endosperm starch in Thorne, Iowin, and Pilot 13 wheat at 4, 5, and 9 days after pollination, respectively. Brenchley (1909) first observed starch in wheat endosperm 10 days after pollination, and Percival (1921) described its first appearance in this region within 12 to 16

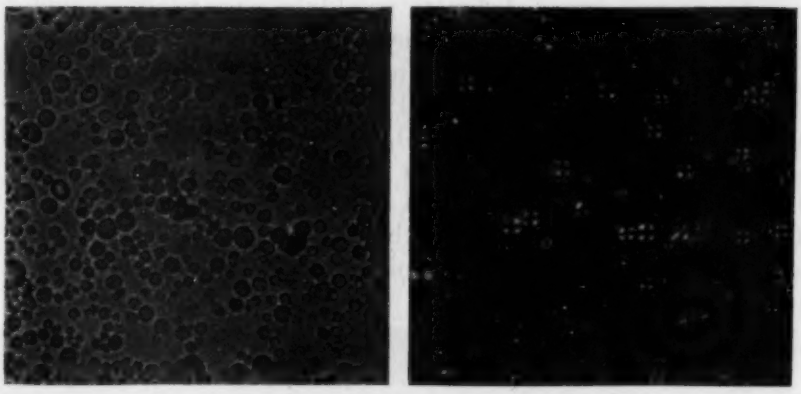

Fig. 1. Left: Starch from Thorne wheat 2 days after pollination. Right: The same field between crossed Nicol prisms. Magnification 500 X.

days. Climatic conditions probably have considerable influence upon the time of initial deposition of starch. Varietal differences may also be involved.

Processing difficulties were encountered in separating starch from wheat which had been harvested from 2 to 12 days after pollination. After 12 days, however, the ordinary laboratory method yielded starch of high quality, which had good color, odor, and flavor, and was relatively free from granule injury. In the purification of starch, advantage is usually taken of the different sedimentation rates of starch and other types of particles, separation of the former being favored by sharp differences in size and density. Before 12 days after pollination it was difficult to separate the chloroplasts from the wheat starch granules. This was partly due to the comparatively small size and slow settling rates of the latter. Under these conditions the starch sample was greenish colored and had high nitrogen content (see Table II). Similar difficulties were encountered in attempting to separate starch from corn soon after pollination.

The relation between starch content of wheat grain and degree of maturity of the grain appears to be linear. About the same amount of starch apparently is produced daily from the time of the first formation of starch in the endosperm until the maturity of the grain. This is perhaps not too surprising. At the time of pollination, vegetative growth of the wheat plant is essentially complete and is subsequently strongly subordinated to development of the grain or caryopsis. From this time on, therefore, the major portion of the carbohydrate material produced photosynthetically is available for the production of starch. It is reasonable to expect that the amount of starch precursor entering the wheat grain during this latter period would not vary appreciably

from day to day, provided climatic conditions were relatively uniform. Rapid polymerization of the precursor in the grain would thus lead to a regular increment in starch content. This view is based upon the comprehensive data of Thatcher (1913). It is supported by the work of Brenchley (1909), Belval (1924), Miller (1939), and Koblet (1940) on the whole grain. The regular increase in the amount of starch obtained from Thorne, Iowin, and Pilot 13 wheat at successive stages of maturity is also in accord with this view, since the amount of starch separated is usually a relatively uniform fraction of the starch present

Fig. 2. Starch from Thorne wheat 8 days after pollination. Note smooth, transparent granules, sometimes bean-shaped. Birefringence of small granules is equal to that shown in Figure 1. Magnification 595 ×.

in the grain. Miller's (1939) data also indicate a decrease in starch content of the grain that may correspond to the decrease in starch yield from Thorne and Iowin wheat between 32 and 41 days after pollination and from Pilot 13 wheat between 28 and 32 days after pollination. If the decrease in yield is, indeed, due to an actual decrease in starch, and not merely reflective of processing difficulties, the cause of the decrease remains to be determined.

In general, granule size of the starches separated increased until about 16 days after pollination, after which time relatively little change occurred. Unripe wheat was reported by Schmorl (1936) to have a 1 to 10.8 ratio of large to small starch granules. The corre-

sponding ratio in ripe wheat was 1 to 4.8. Teller (1938) also observed increase in granule size in developing wheat grain. He noted that the first granules formed were very small, and the next were larger and crescent shaped, often with a projection upon the inner side. The crescent forms gradually filled in, taking the shape of normal wheat starch granules. Similar shapes and sizes were observed during the early stages of the present study (Figure 2). Up to approximately 16 to 20 days after pollination, the granules appeared very transparent and lacking in detail, but with increasing maturity they looked more dense, although still transparent, while at the same time they showed folds and indentations, and in some cases exhibited lamellation (Figure 3).

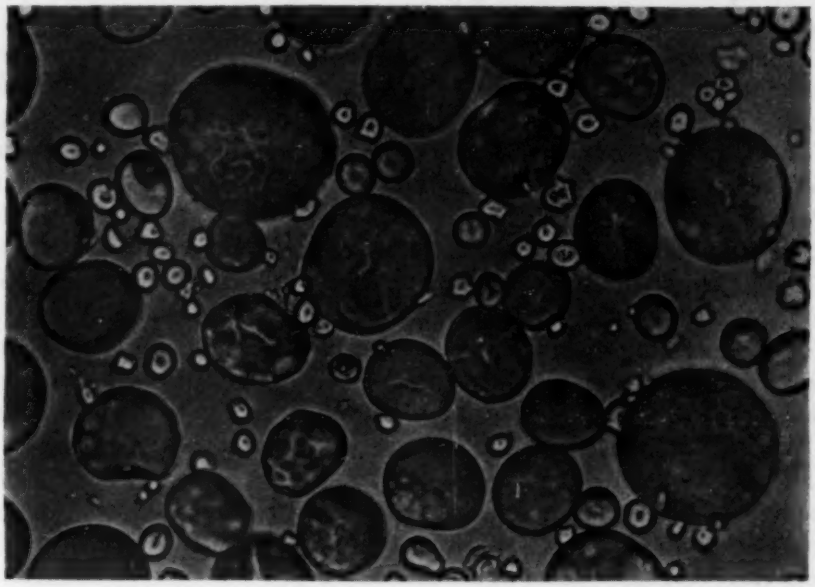

Fig. 3. Starch from Thorne wheat 32 days after pollination. Note surface markings on granules, and larger average size than that of granules shown in Figure 2. Magnification 595 X.

The indentations were probably attributable to the pressure of small granules against the larger ones in endosperm cells crowded with starch and protein although enzymic action may have played a part in their formation. In starch samples that were prepared between the sixteenth day after pollination and maturity, the fluctuations in the percentages of granules falling into different size groups (Table II) cannot be accounted for at present. They may have been due to unobserved variations in processing.

At all stages that were studied, the starch granules were birefringent and gave a blue color with iodine. Young (1938) and Evans (1941) reported similar observations on starch granules from immature beans

and corn respectively. Maige (1922), however, found that young bean starch granules stained red brown with iodine. Very young *Narcissus* starch granules were observed by Badenhuizen (1939) to stain blue with iodine but to have no birefringence. Lampe (1931) found no birefringence in undried granules from immature corn. Conflicting statements with respect to coloration of young starch granules with iodine may be due to the variations in shade and intensity of color exhibited by granules treated with different concentrations of the reagent. Inadequate illumination may account for the failure of some workers to observe birefringence, since a strong light is required to show it in very small granules. There is, of course, the further possibility that young granules of different species of starches have unlike characteristics.

The comparative uniformity in phosphorus, nitrogen, and ash content of the starch from the three different varieties of wheat and from kernels 12 days after pollination to maturity is quite striking. The slight increase in phosphorus content of starch from Pilot wheat between 16 and 24 days after pollination may not be significant. Possible variations occurring in the composition of starch up to 12 days after pollination were obscured by the impurity of the starch. These results, in general, amplify and confirm data reported by Mangels (1934).

The most interesting difference found in the starches was an increase in the amylose content between 8 days and 24 days after pollination as determined by sorptive capacity of the starches for iodine. This increase from about 19% on the eighth to twelfth day to about 25% on the twenty-fourth day was continuous and was observed in each of the three varieties of wheat. On or subsequent to the twenty-fourth day after pollination, the ratio of amylose to amylopectin was relatively constant, being approximately 1:3. The data on the iodine sorptive capacity and calculated percentage of amylose present in the various starches are shown in Table III. These data are tentative in that it is assumed that contamination, if any, by nonstarchy material was equal at all stages. That the peripheral cells of the endosperm, especially those in the cheeks or flanks of the kernel, are the first to contain starch and that the granules formed here are relatively small have been shown by Percival (1921). The present data suggest the possibility that the granules laid down in the earlier stages of the development of the kernel, i.e., largely in the peripheral areas, are richer in amylopectin than the granules later formed in the endosperm tissues nearer the embryo. Apparently the difference is not one between small and large granules. A small-granule fraction of Pilot 13 wheat starch separated 53 days after maturity had essentially the same

iodine sorptive capacity as the whole starch. Whether the variation in amylose to amylopectin ratio is primarily related to time of deposition or to granule size, it appears that the granules in the peripheral portions of the endosperm are comparatively the richest in amylopectin.

Viscosity of the starch was not found to vary with maturity or variety of wheat. In 2% concentration, relative viscosity for practically all the starches was 1.4; in 5.5% pastes it ranged from 2.1 to 2.5. These observations do not agree with the specific varietal viscosities reported in the literature, of which those given by Mangels (1934) may be taken as representative. He, however, used chemical gelatinizing agents, higher concentrations of pastes, and a different type of viscometer, and determined viscosity at 30°C. Lack of agreement is not uncommon between viscosity data obtained under such widely different conditions.

The light transmittancy curve of a paste of wheat starch from young grain (12 to 16 days after pollination) differed from that of starch from mature grain (55 to 60 days after pollination) in height of base line, point of inflection, and general form (Figure 4). The amount of light transmitted by ungelatinized starch from the immature wheats was greater than that from ungelatinized starch from the mature wheats. This indicates that the former granules are more transparent, a conclusion which is in accord with microscopic observations. As the gelatinization temperature was approached, immature starch gave a sharp decrease to a minimum at 61° to 63°C, followed by a rapid increase, while mature starch merely showed an increase in translucency, beginning at about 55° to 60°C and rising rapidly. Differences occurring in translucency curves above 70°C could not be correlated either with variety or maturity of wheat.

It may be concluded, in general, that aside from the possible change in the ratio of amylose to amylopectin, there is surprisingly little difference between starch from immature wheat and that from the corresponding mature grain. This may be of considerable interest to those who must use wheat that is harvested before full maturity because of various conditions such as insect attack and damage by hail or frost. The influence may be local or comparatively widespread. In the latter case, in spite of harvesting and storage problems, considerable quantities of slightly immature wheat may come upon the market. There is no apparent reason, from the standpoint of starch characteristics, why this wheat could not be used for industrial purposes.

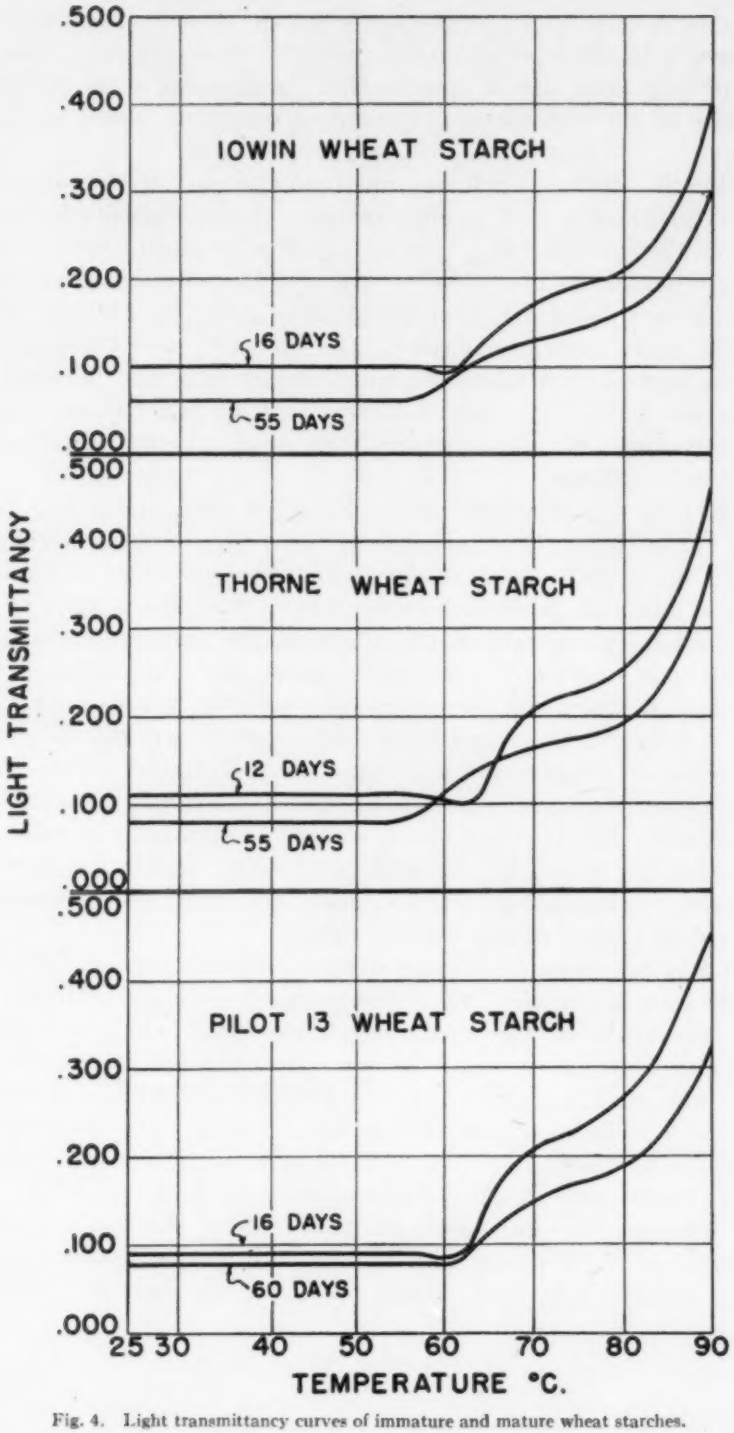

Fig. 4. Light transmittancy curves of immature and mature wheat starches.

Summary

Starches were prepared and studied from Thorne soft red winter, Iowin hard red winter, and Pilot 13 hard red spring wheat at several stages of maturity during one crop year, from shortly after pollination to about 3 weeks after normal harvest. Endosperm starch was abundant in Thorne wheat 8 days after pollination. From this time to maturity, the increase in amount of starch obtained upon processing was approximately a linear function of time. Increase in starch-granule size was marked up to 12 or 15 days after pollination, but there was relatively little subsequent change. In the earlier samples, the granules appeared smooth and very transparent. With increasing maturity of the wheat, they gradually became somewhat less transparent and showed lamellation and surface irregularities. At all stages studied, the granules were birefringent and were colored blue by iodine solution.

Nitrogen, phosphorus, and ash content of the starch were not found to change materially as the wheat matured. Viscosity of the starch paste, likewise, was not found to be affected. Dissimilarities were found in light transmittancy curves that were obtained during pasting of starch from very young and from mature wheat. Starches from the different varieties of wheat were also found to give unlike light transmittancy curves.

As the wheat matured there was an apparent increase in the amylose-amylopectin ratio as shown by the variation in the sorptive capacity of the starch for iodine. This variation may indicate a relatively more rapid synthesis of amylopectin in the earlier stages of deposition of starch in the endosperm than in the later stages. On this basis, it would be anticipated that starch from the outer portion of the endosperm in the cheeks of the kernel would be relatively richest in amylopectin, since that is the area first filled with starch.

From the standpoint of starch characteristics reported here, immature wheat should be acceptable for commercial use.

Acknowledgment

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Literature Cited

Badenhuizen, N. P.
1939 Growth and corrosion of the starch grain in connection with our present knowledge of the microscopical and chemical organization. Protoplasma **33**: 440–468.

Bates, F. L., French, D., and Rundle, R. E.
1943 Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65**: 142–148.
Belval, H.
1924 La genèse de l'amidon dans les céréales. Rev. gén. botan. **36**: 308–324, 337–356, 395–411.
Brenchley, W. E.
1909 On the strength and development of the grain of wheat (*Triticum vulgare*). Ann. Botany **23**: 117–138.
—— and Hall, A. D.
1909 The development of the grain of wheat. J. Agr. Sci. **3**: 195–217.
Evans, J. W.
1941 Microscopic examination of developing corn starch. Cereal Chem. **18**: 462–464.
Koblet, R.
1940 Untersuchungen über die stofflichen Veranderungen im wachsenden und reifenden Weizenkorn. Ber. schweiz. botan. Ges. **50**: 90–232.
Lampe, L.
1931 A microchemical and morphological study of the developing endosperm of maize. Botan. Gaz. **91**: 337–376.
MacMasters, M. M., and Hilbert, G. E.
1944 Stored and damaged wheats for starch production. Cereal Chem. **21**: 258–266.
1944a Glutinous corn and sorghum starches. Ind. Eng. Chem. **36**: 958–962.
Maige, A.
1922 Influence de la température sur la formation de l'amidon dans les cellules végétales. Compt. rend. soc. biol. **86**: 685–686.
Mangels, C. E.
1934 Varietal and regional variations in properties of wheat starches. Cereal Chem. **11**: 571–585.
Miller, E. C.
1939 A physiological study of the winter wheat plant at different stages of its development. Kans. Agr. Expt. Sta. Tech. Bull. 47.
Morgan, W. L.
1940 Pasting and identification of starches. Ind. Eng. Chem. (Anal. Ed.) **12**: 313–317.
Percival, J.
1921 The Wheat Plant. Duckworth and Co., London.
Schmorl, K.
1936 Studien über Reifevorgänge am Brotgetreide (Wz.). Mühlenlab. **6**: 11–15.
Teller, G. L.
1938 Plant diastase in evidence as to the formation and structure of starch granules. Plant Physiol. **13**: 227–240.
Thatcher, R. W.
1913 The progressive development of the wheat kernel. J. Am. Soc. Agron. **5**: 203–213.
1915 The progressive development of the wheat kernel. II. J. Am. Soc. Agron. **7**: 273–282.
Young, P.
1938 Starch formation in the leucoplasts of *Phaseolus vulgaris*. Bull. Torrey Botan. Club **65**: 1–8.

GRAIN STORAGE STUDIES. I. INFLUENCE OF LOCALIZED HEATING OF SOYBEANS ON INTERSEED AIR MOVEMENTS [1]

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The heating of grain in bulk usually starts in small pockets of high moisture. The low heat conductivity of the grain tends to prevent the loss of the heat produced, with the result that rapid local increases in temperature may occur with concomitant deterioration in grain quality. The mechanism of the spreading and migration of heating zones in grain bins has been the subject of considerable speculation. Ramstad and Geddes (1942) suggested that strong interseed air movements may occur in heating grain. They used a probing method to sample the interseed air and to determine the temperature in a bin of damp soybeans, but were not able to relate the temperature increases to the carbon dioxide concentrations which were found to exist. They therefore assumed a movement of this gas away from the regions of heating. These workers suggested that such interseed air movements might result in a translocation of moisture in the vapor phase from warm to cool portions of the grain bulk, thus promoting respiratory and heating tendencies in the latter. Kiesel, Vassilieva, and Tsygankova (1939) had demonstrated that the application of temperature differentials across small masses of wheat (6 liters), initially at a moisture content where spoilage was not to be expected, could result in moisture translocation from the heated toward the cooled end to an extent sufficient to cause deteriorative changes in the seeds in the cooled zone. These laboratory-scale findings were recently confirmed by Anderson, Babbitt, and Meredith (1943) who worked with a temperature differential of 35°C across 6 feet of grain and concluded that the transfer of moisture was due to convection currents.

Howe (1943) investigated the interseed air and moisture changes in a small bin of wheat infested with insects and suggested that warm spots in a grain bin would set up convection currents which tend to prevent the accumulation of carbon dioxide and to renew the oxygen supply around the heating seeds. Oxley (1944), in a study of the

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spread of heating areas in bulk grain, listed several physical factors which contribute to this phenomenon. Among these are the thermal conductivity of the grain, convection currents of air through the interseed spaces, water vapor movement involving heat transfer due to latent heat of vaporization, and radiation.

The present study was undertaken to secure more reliable information on interseed gas movements than was possible by the probing methods used by Ramstad and Geddes (1942). These workers obtained information only on regions to 50 feet in depth in a bin containing 97 feet of grain; furthermore, they found it difficult to maintain a vertical direction for the path of the probe once it was driven below the grain surface. Thus they could not ascertain whether at any maximum depth the probe was near the center or a sidewall of the bin, nor did they know whether the same region was being sampled from one time to the next. In the present study, several series of pipes were installed in fixed positions before a bin was filled with grain, making it possible to follow the temperature and interseed air composition in fixed locations over a considerable period of time.

Experimental Procedure and Results

A permanent means of sampling interseed air and temperature in an interstitial, open-top, concrete bin was provided by arranging four clusters of small diameter iron pipe of different lengths along a cross section of the bin, the pipes in each cluster opening at successive 10-foot intervals (Figure 1). Each pipe was suspended from a heavy 10-inch steel I-beam supported by the concrete walls of adjacent bins. The longest pipe (90 feet) was $\frac{3}{8}$ inch in diameter, allowing for the introduction of a thermocouple attached to a wire lead so that the temperature at any point in the 90-foot length could be taken, as well as providing for sampling of the interseed air at the 90-foot depth. The other pipes in each cluster were $\frac{1}{8}$ inch in diameter and served for the withdrawal of interseed air from the various predetermined zones. Gas samples were taken with a hand-operated piston pump which created suction in a train comprising the pipe, gas sample tube, liquid-displacement volume-measuring device, and pump, respectively. An amount of air at least three times the calculated internal volume of each pipe was withdrawn through the sample tube before the gas sample was taken. The maximum volume of air withdrawn ($\frac{3}{8}$-inch pipe, 90-foot length) was 11.1 liters. The air samples were analyzed for carbon dioxide and oxygen by means of a Haldane-Henderson gas analysis apparatus.

The bin was filled with 10 carlots of soybeans totaling 971,860

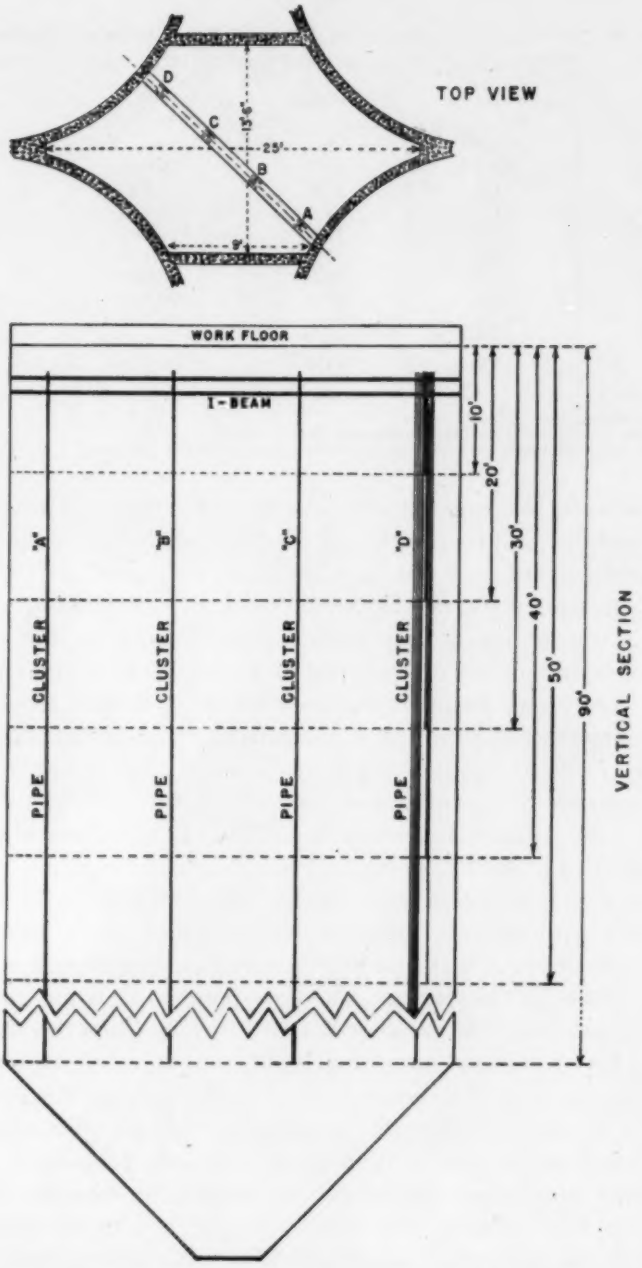

Fig. 1. Diagram of the top and a vertical section of the commercial elevator bin showing arrangement of pipes for determining the temperature and sampling the interseed air.

TABLE I

RECORD OF SOYBEANS[1] PLACED INTO BIN FOR BULK STORAGE EXPERIMENT

Date 1942	Moisture[2]	Damage[3]	Weight	Depth, top of bin to top of load
	%	%	*lb*	*feet*
11–13	12.4	13.0	75,000	4
11–13	14.8	11.0	113,580	12
11–12	13.2	17.2	88,440	25
11–12	12.2	21.6	123,000	34
11–12	14.0	12.4	95,100	46
11–12	14.6	25.0	90,000	55
11–9	16.8	10.8	99,450	65
11–4	14.4	20.2	91,730	75
11–3	12.3	19.7	110,640	83
11–2	11.9	11.3	84,920	94

[1] All lots were Sample Grade.
[2] Moisture determined with Tag-Heppenstall electric moisture meter.
[3] Percentage of damaged beans is that portion which is green and immature.

pounds between November 2 and November 13, 1942. The characteristics of each lot and its position in the bin are given in Table I.

The grain was of poor commercial quality owing to early September frosts. Subsequent warm weather before harvesting caused drying of this green and immature crop to normal moistures, so that difficulty was encountered in selecting grain of higher than normal moisture content. Only one lot, that between 65- and 75-foot levels, had a moisture content (16.8%) which previous experience had shown to be definitely unsafe for storage. The grain temperatures and the carbon dioxide contents of the interseed atmosphere for each sampling zone at various time intervals over a period of 112 days are represented graphically in Figure 2.

Owing doubtless to the low average moisture content of the grain and the low atmospheric temperatures prevailing, little indication of heating or undue concentrations of interseed carbon dioxide was noted until after 80 days of storage. The only point of heating appeared in the D section (near the south sidewall) 70 feet from the top of the bin in the layer of grain containing 16.8% moisture. That the spread of this heating area was more marked in an upward direction than downward is evident from the temperature values surrounding this zone. This upward spread of heating probably followed a path of water vapor movement carried by air convection currents from the hot area to the colder regions above it. Such a trend would be in accord with the laboratory studies of Kiesel, Vassilieva, and Tsygankova (1939) and Anderson, Babbitt, and Meredith (1943). It is notable that in regions of the grain bulk most remote from D-70, the grain actually cooled throughout the period of storage, except for a

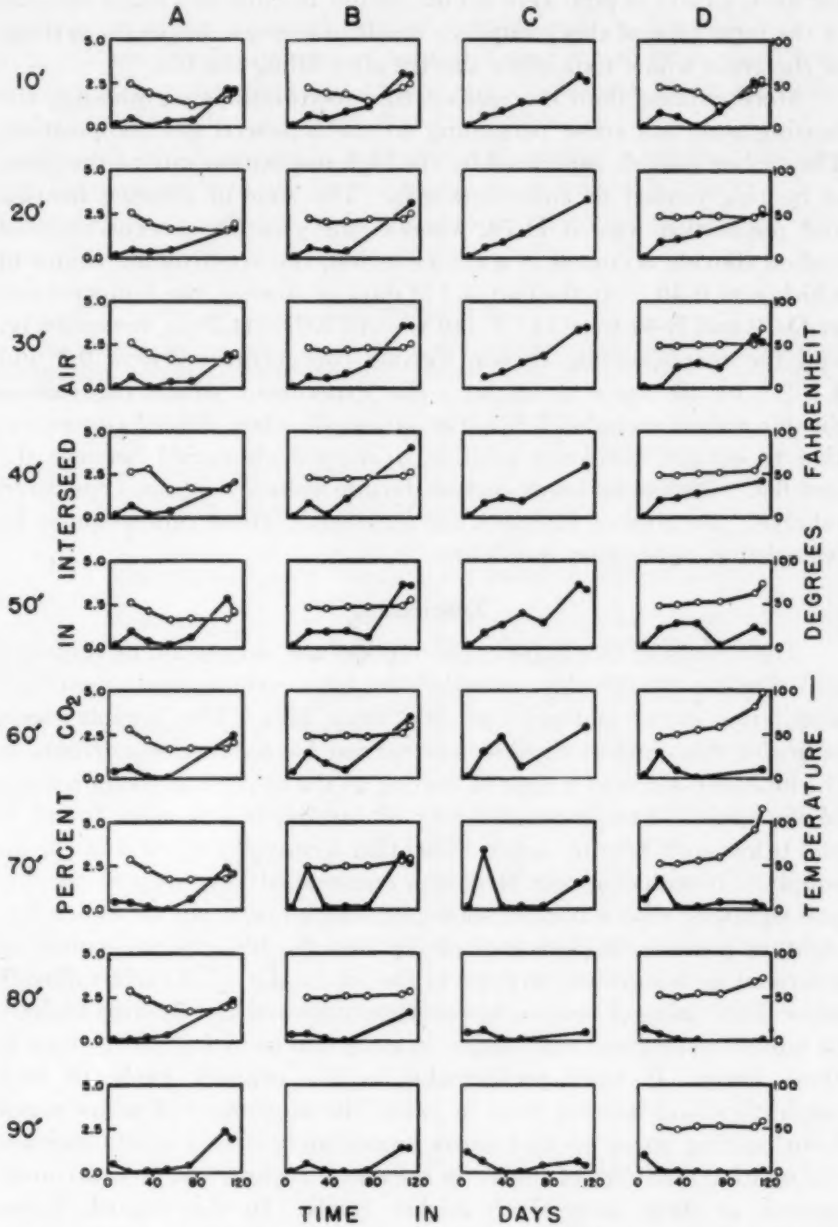

Fig. 2. Changes in temperature and interseed carbon dioxide concentration in soybeans in bulk storage. Temperature readings are represented by open circles and carbon dioxide values by solid circles.

very small rise during the last few days. Unfortunately, temperatures for the C cluster of pipe were not obtainable because of a slight twisting of the large pipe of this group as a result of stresses due to the settling of the grain which took place shortly after filling the bin.

More striking than the temperature relationships surrounding the heating zone are those pertaining to the interseed gas composition. The carbon dioxide generated by the high respiration rate at the point of heating tended to move upwards. The zone of greatest heating and respiration was at D-70, whereas the greatest concentration of carbon dioxide occurred in a colder region, the approximate center of which was B-40. At the end of 112 days of storage, the temperatures at D-70 and B-40 were 115°F (46°C) and 53°F (11.7°C), respectively, and the corresponding carbon dioxide concentrations were 0.2 and 4.1%. In all zones throughout the experiment where the carbon dioxide values exceeded 0.5%, the ratios of carbon dioxide concentration to oxygen deficiency relative to normal air varied between 0.4 and 0.7. Ratios for lower carbon dioxide values were not considered reliable. Because of interseed air movement, these ratios cannot be regarded as respiratory quotients.

Discussion

The results of this experiment support the suggestion of Ramstad and Geddes (1942) that considerable interseed air movement and ventilation occur in large open-top grain bins. The present study indicates that carbon dioxide was carried by convection currents in the interseed air from a zone of heating to the upper and cooler regions of the grain. Low concentrations of carbon dioxide were found in and below such heating zones, indicating a constant renewal of the air supply. It would appear that in a commercial bin, open at the top and equipped with a bottom slide gate which might not be sufficiently tight to prevent the entrance of air into the bin, the movement of interseed air is sufficient to prevent the accumulation of carbon dioxide in localized areas of heating in concentrations sufficiently high entirely to inhibit respiration and further heating due to biological agencies in these areas. If mold proliferation is the primary cause of high respiratory and heating rates in grain, the movement of water vapor from heating zones to cool areas immediately above would increase the relative humidity of the air in the cooler regions and promote mold growth at these successively higher levels. In this regard, Rozsa (1935), Galloway (1935), Haines (1937), and Smith (1938) have indicated that atmospheric humidity rather than substrate moisture governs the germination and proliferation of mold fungi.

Summary

Changes in the interseed air composition and the temperature of soybeans stored throughout a winter season in a large open-top commercial bin were followed at numerous points in the grain bulk by the aid of a series of preinstalled small diameter pipes.

Heating, which started in a zone of high moisture, led to considerable air movement in the stored grain, as indicated by appreciably higher carbon dioxide values in the upper and cooler portions of the grain than in, or below, the zone of heating.

Acknowledgment

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Literature Cited

Anderson, J. A., Babbitt, J. D., and Meredith, W. O. S.
1943 The effect of temperature differential on the moisture content of stored wheat. Can. J. Research C **21**: 297–306.

Galloway, L. D.
1935 The moisture requirements of mould fungi, with special reference to mildew in textiles. J. Textile Inst. (Transactions) **26**: T123–129.

Haines, R. B.
1937 Microbiology in the preservation of animal tissues. Dept. Sci. Ind. Research (Brit.). Food Invest. Board Special Report No. 45.

Howe, R. W.
1943 An investigation of the changes in a bin of stored wheat infested by insects. Bull. Entomol. Research **34**: 145–158.

Kiesel, A., Vassilieva, N., and Tsygankova, G.
1939 Translocation of moisture in the bulk of stored grain. Compt. rend. acad. sci. U.R.S.S. (N.S.) **24**: 786–790.

Oxley, T. A.
1944 The properties of grain in bulk. III. The thermal conductivity of wheat, maize and oats. J. Soc. Chem. Ind. **63**: 53–55.

Ramstad, P. E., and Geddes, W. F.
1942 The respiration and storage behavior of soybeans. Minn. Agr. Expt. Sta. Tech. Bull. 156.

Rozsa, T. A.
1935 Drinking habits of the wheat berry. Northwestern Miller, Production Annual **184** (2): 73–74.

Smith, G.
1938 An Introduction to Industrial Mycology. Edward Arnold and Co. Ltd., London.

GRAIN STORAGE STUDIES. II. THE EFFECT OF AERATION, TEMPERATURE, AND TIME ON THE RESPIRATION OF SOYBEANS CONTAINING EXCESSIVE MOISTURE [1]

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The respiration of dormant seeds as estimated by laboratory techniques has been shown to be closely related to the tendency of grain to spoil and heat when stored in bulk under unfavorable moisture conditions. The factors affecting grain respiration have been exhaustively studied by Bailey and Gurjar (1918) and Bailey (1921, 1940), who incubated quantities of grain of known moisture content for a fixed period (usually four days) at constant temperature, and determined the accumulated carbon dioxide due to respiration by absorption of the gas into standard caustic or baryta solutions. This technique was applied in studies of the effect of moisture content and other variables on the respiration of the cereal grains and flaxseed. Coleman, Rothgeb, and Fellows (1928), Swanson and Fenton (1932), Larmour, Clayton, and Wrenshall (1935), Ramstad and Geddes (1942), and others have used this method in grain respiration investigations.

It has long been recognized that grain respiration is depressed by accumulation of carbon dioxide in the interseed air. In this regard, Larmour *et al.* (1935) showed that increased air space in the respirometer, as well as more frequent aspiration, resulted in greater carbon dioxide production in samples of high respiratory activity. In order to reduce the possible inhibition of respiration by carbon dioxide accumulation, Ramstad and Geddes (1942) adjusted the sample weights (hence the free air space) and the incubation period so that the carbon dioxide production did not exceed an arbitrary maximum value.

It seems probable that the static technique, involving measurements of carbon dioxide production over a fixed period of two to four days, has frequently failed to reveal the maximum respiratory potential, particularly of high-moisture samples. In such samples the danger of respiratory depression is more probable. Moreover, in view of the paramount importance of molds in the over-all respiration of

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grain indicated by the studies of Larmour *et al.* (1935) and of Ramstad and Geddes (1942), the respiratory rate would be expected to increase with time under storage conditions which would permit of mold proliferation.

The widespread use of the static method was doubtless due in part to its simplicity and also to the assumption that the experimental conditions were analogous to those which exist in grain in bulk storage. However, it has been shown by Howe (1943), Oxley (1944), and Milner and Geddes (1945) that interseed air movements occur in the heating of grain in bulk which tend to prevent the accumulation of inhibitory concentrations of carbon dioxide in localized areas where heating and rapid respiration are taking place. Thus, studies of the relative respiratory activity of grain at different rates of aeration may be of considerable practical significance.

In the majority of respiration studies at moistures within the range which may be encountered in bulk storage, simultaneous measurements of the oxygen and carbon dioxide content of the interseed air have not been made. Most workers have assumed that carbohydrates are the principal materials oxidized. This assumption has not been critically examined in the case of dormant seed respiration.

Microtechniques for respiration measurements on samples as small as a single seed have become increasingly popular in recent years. These methods, using the Warburg apparatus or its modifications, have been employed by Shirk and Appleman (1940) for wheat, and by Taylor (1942) for wheat and rice. Stiles and Leach (1931) and Leach (1932) have adapted an apparatus known as a katharometer to such studies. A major difficulty in most microtechniques is that simultaneous measurements of carbon dioxide production and oxygen uptake cannot be made on the same sample without terminating or interrupting the conditions within the respiration vessels at rather short intervals. Furthermore, in view of the variety of microorganic species which are normally present in varying numbers and which may contribute to the respiratory activity (Milner, Warshowsky, Tervet, and Geddes, 1943, and Tervet, 1945), data for the respiration of one or a few seeds are of dubious value as an index of the behavior of the seed in bulk.

In connection with a study of the factors affecting the respiration of soybeans, the considerations previously outlined required the development of a laboratory technique which would give the maximum respiratory potential of the grain and provide simultaneous measurements of oxygen consumption and carbon dioxide production. A further object was to study the influence of aeration rate, interseed carbon dioxide concentration, temperature, and time on the respiratory

rates and respiratory quotients of soybeans at a moisture content conducive to deterioration in storage.

Apparatus and Methods Employed for Respiration Measurements

Apparatus. It was deemed necessary to construct an apparatus which would possess the following features: (1) employment of seed samples of sufficient size to minimize sampling and experimental errors, (2) provision for continuous aeration at a series of constant preselected rates, (3) maintenance of the samples under constant selected relative humidity and temperature, (4) collection and measurement of the entire effluent air from the respirometers at regular time intervals, (5) sampling and analysis of the effluent air for oxygen and carbon dioxide content, and (6) continuous and dependable operation for extended periods.

The apparatus used to determine the respiratory characteristics of seed samples (Figure 1) allowed for the continuous movement of fresh air to the seed samples which were maintained at constant temperature and humidity. The principle of operation is as follows:

Outside air, freed from carbon dioxide by passage through soda-lime in bottle A, is passed through a humidifying solution (sulfuric acid or saturated salt solutions) in bottle B, and then through the seeds in the respirometer vessel C. The humidifying solution, as well as the seed sample, is maintained at constant temperature in a thermostatically controlled water bath M. A uniform rate of air flow through the humidifying solution and the seed sample is maintained by the gradual lowering of a calcium chloride solution (Sp.G. 1.40), in which carbon dioxide is only very slightly soluble, in the graduated spirometer E of 2000 ml capacity. Air is drawn through the seed samples into the spirometer by the slow descent of a straight-sided 2000 ml leveling bulb F, which is connected with the spirometer. The rate of descent of the leveling bulb is governed by the rotation of pulley P, about which a stout cord attached to the leveling bulb is looped and secured. Pulley P is, in turn, fastened by set-screws to lineshaft L which is driven at constant speed by a synchronous motor reduction gear unit U and chain R. The speed of this unit, as well as the diameter of pulley P, is so adjusted that a maximum volume of 2000 ml of air can be drawn through the respiring seeds in the course of 23.5 hours. Pulley P also has steps of a smaller diameter so that in addition to the 2000 ml rate, air volumes of 1500, 1000, 750, 500, and 250 ml can be passed through the respiring seeds.

With such a system, spirometer E accumulated all of the air drawn through the respiring sample in a one-day period. Each daily accumulation period is ended by stopping the apparatus for 30 minutes and

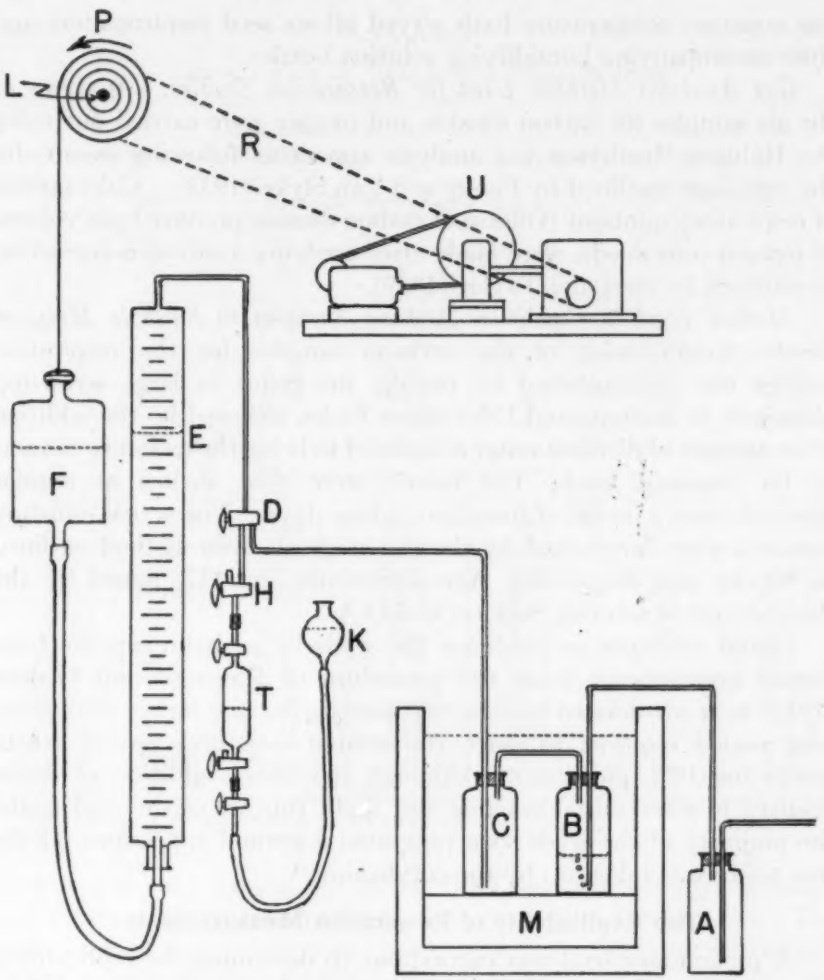

Fig. 1. Diagram of apparatus used to determine the respiratory rate of seeds at constant temperature.

withdrawing a sample of this air into gas sample tube T for analysis by means of stopcocks D and H and mercury leveling bulb K. To begin the next day's trial, the balance of the air in spirometer E is discharged to the atmosphere by rewinding the cord suspending the leveling bulb onto pulley P and manipulating stopcocks D and H. Pulley P is then adjusted so that the surface of the retaining liquid in spirometer E is at the zero point of its calibration when the spirometer is again in communication with the seed sample. The regulating mechanism U is then placed in continuous operation for a further 23.5 hour period.

The lineshaft served to control six individual respirometer systems, thus permitting the simultaneous testing of six seed samples. The

one constant temperature bath served all six seed respirometers and their accompanying humidifying solution bottles.

Gas Analysis Methods Used for Respiration Studies. Analyses of the air samples for carbon dioxide and oxygen were carried out using the Haldane-Henderson gas analysis apparatus following essentially the technique outlined by Peters and Van Slyke (1932). Calculations of respiratory quotient (volume of carbon dioxide produced per volume of oxygen consumed), were made after applying a nitrogen correction as outlined by Best and Taylor (1939).

Method Used to Condition Soybean Samples to Suitable Moisture Levels. Conditioning of the soybean samples for the respiration studies was accomplished by placing the beans in large screw-top glass jars, or in stoppered Erlenmeyer flasks, followed by the addition of an amount of distilled water calculated to bring the moisture content to the required level. The vessels were then shaken at regular intervals over a period of from two to four days. The actual moisture contents were determined by the two-stage air-oven method outlined in Service and Regulatory Announcements No. 147, issued by the Agricultural Marketing Service, U.S.D.A.

Initial attempts to condition the seeds by moisture uptake from humid atmospheres using the procedure of Ramstad and Geddes (1942) were abandoned because the samples became moldy during the long period required to reach the desired moisture content (three weeks for 18% moisture). Although the direct addition of water resulted in some initial swelling and slight rupture of the seed coats, the majority of the seeds soon presented a normal appearance as the free water was taken up by the cotyledons.

The Replicability of Respiration Measurements

A preliminary trial was carried out to determine the replicability of respiration measurements on soybeans. A sound sample of Illini soybeans of the 1942 crop, testing 94% germination, was used. This sample was conditioned to a moisture content of 18.4% and subsamples weighing 300 g were placed into six respirometer bottles which were assembled in the water bath and connected to outside air lines in series with bottles containing saturated potassium chloride solution (85% relative humidity at 25°C). The data of Ramstad and Geddes (1942) relating the equilibrium moisture content of soybeans in contact with atmospheres of known relative humidity and the data of Spencer (1926) for the relative humidity of air in equilibrium with various saturated salt solutions were used in selecting this humidifying solution.

Respiration trials were first made at a temperature of 20°C and approximately 1950 ml of daily aeration, but the respiration values

TABLE I

REPLICABILITY OF RESPIRATION MEASUREMENTS

Days	Mean values for six replicates				Respiratory rate[1]	
	Daily aeration	Interseed CO_2 concentration	Respiratory quotient	Rate[1]	Standard error (single detmn.)	Coefficient of variability
	ml	%		*mg*	*mg*	%
TRIALS CONDUCTED AT 20°C						
1	1967	0.46	0.99	7.2	0.03	0.46
2	1929	0.29	0.85	4.5	0.03	0.74
3	1960	0.27	0.99	4.2	0.70	16.7
TRIALS CONDUCTED AT 30°C						
4	1922	0.66	0.77	10.1	0.91	9.1
5	1948	1.00	1.01	15.7	1.85	11.8
6	1952	1.71	0.95	26.6	4.38	16.5
7	1952	2.96	0.75	46.2	7.02	15.2
8	1659[2]	5.12	0.80	67.9	7.10	10.5
9	2024	5.00	0.95	81.0	4.67	5.8
10	2003	4.77	0.97	76.5	4.17	5.5
11	2000	4.53	0.99	72.4	4.08	5.6
12	2003	4.35	1.00	69.7	3.72	5.3
13	2001	4.20	1.00	67.3	4.00	5.9
14	2004	4.04	0.99	64.8	3.60	5.6
15	2004	4.00	1.00	64.1	3.80	5.9

[1] Milligrams carbon dioxide per 100 g dry matter per day.
[2] Power supply failed for 4 hours.

were so low that, at the end of the third day, the bath temperature was raised to 30°C and maintained at this value for 12 days. The mean values for the respiratory characteristics are presented in Table I, together with the standard errors and coefficients of variability for the daily respiratory rates.

The maximum difference in aeration between the six replicate trials was 40 ml. That the small differences which occurred in aeration rate were without material influence on the low rate of carbon dioxide production encountered in this trial is indicated by the data for the fourth day when a power interruption of about four hours caused a daily average aeration of 1659 ml as compared with 1952 ml on the previous day. The carbon dioxide production for the fourth day fell on the smooth curve expressing the results of the 15-day trial.

During the first nine days when the respiration was increasing rapidly, there was a marked variation in the respiratory quotients among the six replicates, but the variability decreased after the respiratory maximum was reached.

The standard errors and the coefficients of variability of the daily respiration values showed that high variability occurred during the phase of rapid respiratory increase (sixth to ninth day). However, when the maximum respiratory level was reached, the replicability was very satisfactory as indicated by coefficients of variability in the order of 5% to 6%. It may be concluded that the most accurate comparisons of the respiratory activity of different samples may be made when equilibrium respiratory conditions are approached.

Influence of Aeration and Temperature on Respiration, Respiratory Quotient, Mold Growth, and Chemical Characteristics

For a study of the influence of aeration at various temperatures with freshly conditioned lots of the same sample, an exceptionally sound sample of Wisconsin Manchu soybean seed was used. This seed tested 94% in germination and only 3% of the seeds were contaminated with microflora. The influence of six aeration rates on the respiration at five temperatures was investigated.

For each series of respiration trials involving a different temperature, a fresh lot of seed was conditioned as previously described. An attempt was made to condition all the samples to 18.5% moisture. Subsamples weighing 250 g were used in each respirometer. The relative humidity of the air streams entering the respirometers was maintained at 85% by sulfuric acid solutions made up according to Wilson (1921).

In addition to aerations of 250, 500, 1000, 1500, and 2000 ml per day, one sample received tank nitrogen at the rate of 250 ml per day. The average analysis of the nitrogen was: nitrogen, 99.79%; oxygen, 0.21%. This small quantity of oxygen made possible a rough approximation of the respiratory quotients.

Upon the completion of each respiration trial, the seeds were analyzed for moisture content and for oil acidity by the method of Zeleny and Coleman (1938). The seeds were also inspected and ranked in the order of the apparent extent of mold proliferation. Detailed mycological examination of the seeds was conducted by surface sterilizing them and incubating on sterile nutrient agar for five days.

The general effect of aeration and time on the respiratory rates at 40°C as shown in Figure 2 is typical for all temperatures studied except that at 45°C where thermal inhibition became a factor. The respiratory data for all temperatures were compared at those points in the respiration curves where approximate equilibrium first appeared. These data are summarized in Table II. The influence of aeration on the respiratory rate at various temperatures is shown in Figure 3.

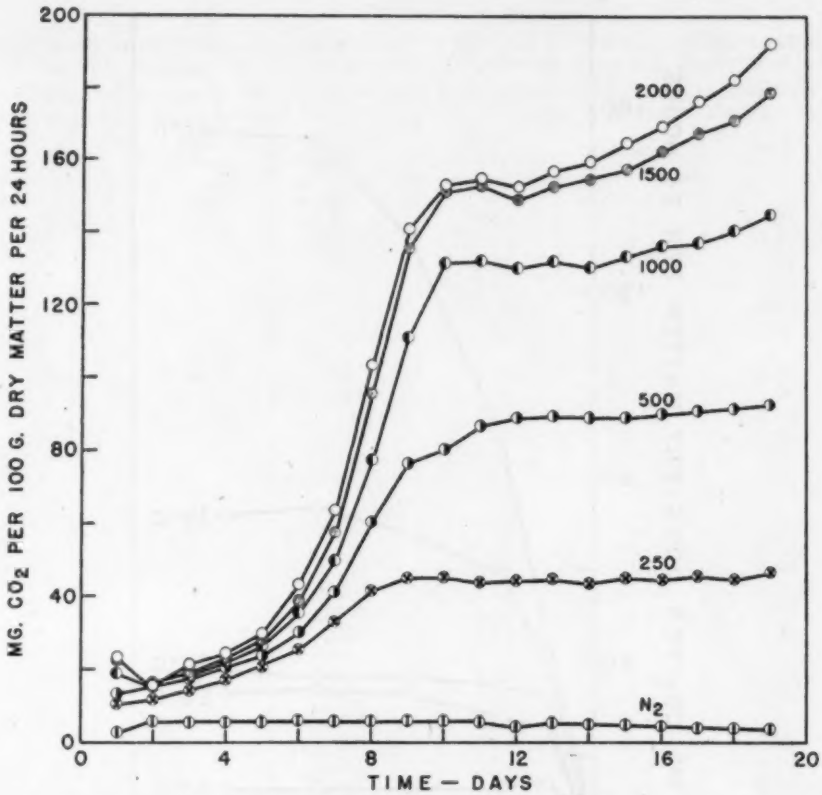

Fig. 2. Influence of time and aeration on the respiratory rate of Wisconsin Manchu soybeans at 19.1% moisture and 40°C. The daily rates of continuous ventilation were: 250 ml of nitrogen (0.21% oxygen) and 250, 500, 1000, 1500, and 2000 ml of air.

Temperature coefficients for intervals of 5°C computed from the respiratory rates shown in Table II are given in Table III.

Under nitrogen, the respiratory activity was extremely low and remained constant for any given temperature over the period of the trial. In contrast, except for a slight decrease during the first few days, the respiration of aerated samples increased slowly, then rapidly, with time, finally leveling off at a value which depended upon the extent of aeration. At the maximum aeration of 2000 ml per day, which was optimal for the trials at 40°C, the respiratory activity was 26 times that under nitrogen. The respiration curves for the aerated samples are analogous in form to microbiological population growth curves. The slight initial decrease in respiration extended over as many as four days and was most marked in the trials at the lower temperatures. This initial decline bore no relation to the subsequent respiratory activity of the seeds.

The data of Table II and Figure 3 show the extent to which aeration must be increased with increasing respiratory activity if maximum

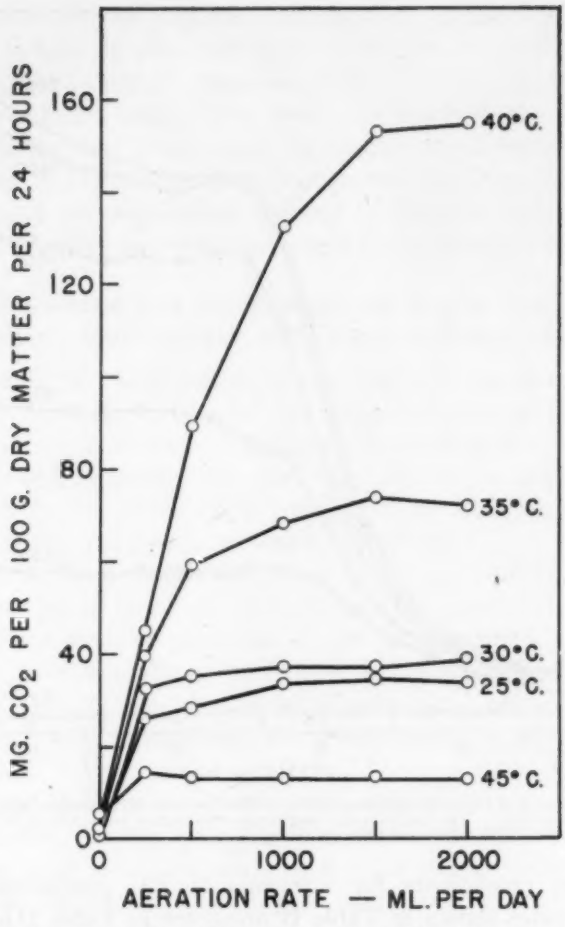

Fig. 3. Influence of aeration on the respiratory rate of Wisconsin Manchu soybeans at high storage moisture at various temperatures.

respiratory values are to be obtained. At 25° and 30°C, all aerations employed appeared to be adequate. However, at 35°C, significant inhibition was evident at an aeration of 1,000 ml per day. While these experiments do not establish the precise interseed carbon dioxide concentrations at which inhibition begins, they do indicate the approximate range within which it occurs. Thus, at 35°C, inhibition became evident when the interseed air contained 12.38% carbon dioxide; at 40°C, inhibitory effects were noted between 10.51% and 13.63% carbon dioxide.

The data in Table II clearly show that the respiratory quotient falls with decreased aeration. This result is in contrast to the report of Mangin (1896) that as the respiration of *germinating* seeds decreases with increasing carbon dioxide concentration, the R.Q. becomes

TABLE II

INFLUENCE OF AERATION, INTERSEED CARBON DIOXIDE CONTENT, AND TEMPERATURE ON THE RESPIRATION OF SOYBEANS AT EXCESSIVE STORAGE MOISTURES

(Data are for days on which approximate respiratory equilibrium was attained)

Temp.	Final moisture	Aeration per day	CO_2 in interseed air	Respiration[1]	R.Q.[2]
°C	%	ml	%	mg	
25.0	18.8	N_2	0.50	1.3	(3.0)
		250	10.55	26.0	0.87
		500	5.78	28.2	0.92
		1000	3.47	33.5	0.92
		1500	2.39	34.6	0.93
		2000	1.74	33.6	1.04
30.0	18.5	N_2	0.23	0.6	(3.8)
		250	13.44	32.5	0.91
		500	7.21	35.2	0.93
		1000	3.83	37.1	0.96
		1500	2.55	37.1	0.95
		2000	2.05	39.7	0.95
35.0	19.3	N_2	0.88	2.1	(8.8)
		250	16.47	39.7	0.85
		500	12.38	59.6	0.94
		1000	7.07	68.1	0.96
		1500	5.12	74.0	1.00
		2000	3.73	71.8	1.00
40.0	19.1	N_2	2.32	5.8	(23.2)
		250	18.65	45.2	0.91
		500	18.50	89.6	0.95
		1000	13.63	132.1	0.95
		1500	10.51	152.8	0.96
		2000	7.98	154.7	0.96
45.0	19.2	N_2	2.25	5.4	(22.5)
		250	6.04	14.6	1.25
		500	2.80	13.5	1.14
		1000	1.38	13.3	—
		1500	0.96	13.9	1.30
		2000	0.68	13.1	1.05

[1] Milligrams CO_2 per 100 g dry matter per 24 hours.
[2] Values for respiratory quotients in brackets, exhibited by samples under nitrogen containing 0.21% oxygen, are only approximate.

TABLE III

EFFECT OF AERATION ON THE ACCELERATION OF RESPIRATION UPON INCREASING THE TEMPERATURE BY INCREMENTS OF 5°C

(Wisconsin Manchu soybeans, initially conditioned to 18.5% moisture)

Aeration rate per day	Acceleration of respiration[1] for temp. interval			
	25°–30°C	30°–35°C	35°–40°C	40°–45°C
ml				
N_2 (.21% O_2)	0.46	3.50	2.76	0.93
250	1.25	1.22	1.14	0.32
500	1.25	1.69	1.50	0.15
1000	1.11	1.84	1.94	0.10
1500	1.07	1.99	2.06	0.09
2000	1.18	1.81	2.15	0.08

[1] Temperature coefficients are based on respiration values for days when approximate respiratory equilibrium was obtained.

TABLE IV

CHANGES IN ACID VALUE OF THE OIL EXTRACTED FROM SOYBEANS AND THE VISUAL CONDITION OF SOYBEAN SEED AFTER RESPIRING UNDER NITROGEN AND VARIOUS RATES OF AERATION

Temperature	Duration of study	Aeration per day	Acid value of oil[1]	Visual condition of seed
°C	*Days*	*ml*	*mg*	
25	19	N_2	0.9	No visible mold.
		250	2.6	Slightly moldy.
		500	3.1	Moldy.
		1000	2.7	Moldy
		1500	3.1	Moldy.
		2000	4.0	Moldy.
30	15	N_2	1.3	No visible mold.
		250	1.4	Degree of moldiness in-
		500	1.4	creases with aeration.
		1000	1.5	
		1500	1.4	
		2000	1.6	
35	25	N_2	4.0	No visible mold.
		250	10.3	Degree of moldiness in-
		500	11.9	creases with aeration.
		1000	15.9	
		1500	14.2	
		2000	14.2	
40	19	N_2	3.4	No visible mold.
		250	6.5	Moldiness in definite
		500	9.3	increasing order with
		1000	13.4	aeration.
		1500	15.4	Seeds slightly browned.
		2000	20.2	
45	11	N_2	3.2	Seeds brown and dis-
		250	3.3	colored; no visible
		500	3.3	molds.
		1000	3.0	
		1500	3.1	
		2000	2.9	
CONTROL—(fresh dry sample)			1.0	

[1] Milligrams of potassium hydroxide required to neutralize the acidity of one gram of oil.

greater. Respiration due to molds on moist dormant seeds may well be the cause of these contrasting results.

Temperature coefficients of respiration for 5°C intervals between 25°C and 45°C shown in Table III increased with aeration in the temperature ranges of 30° to 35°C and 35° to 40°C. No increase in the temperature coefficients with increased aeration was noted between 25° to 30°C or 40° to 45°C. In the latter case, thermal inhibition increased with aeration. At aeration values of 1,000 ml per day and higher, larger temperature coefficients appeared with increasing temperature up to the thermal inhibition range.

Mycological examination at the end of the respiration trials by surface sterilization of the seed and incubation on sterile nutrient agar disclosed that *Aspergillus glaucus* was the principal microorganic contaminant.

The data for the acid value of the oil, together with notes on the visual condition of the seed at the end of the various respiration trials, are given in Table IV. Under nitrogen (where the respiratory activity was minimal), no visible mold growth was evident and no

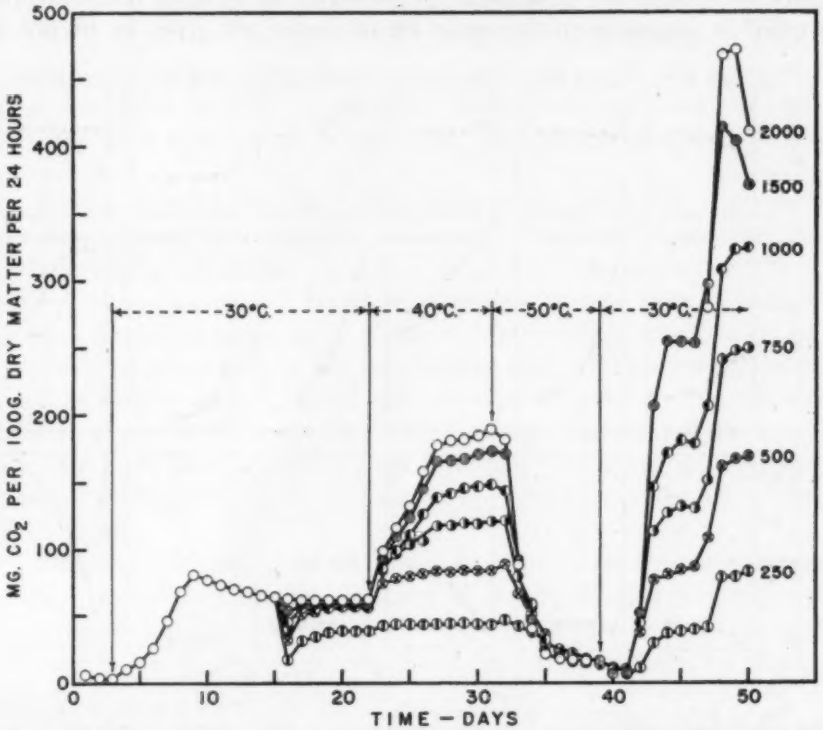

Fig. 4. Influence of time and aeration on the respiratory rate of Illini soybeans at 18.4% moisture, at various temperatures applied in sequence as indicated. The daily rates of continuous aeration were 250, 500, 750, 1000, 1500, and 2000 ml for 300 g of seed sample, up to the 46th day. From the 46th to the 50th day the sample weights were 150 g.

marked change in oil acidity occurred. On the other hand, in the presence of air, mold growth appeared and increased with aeration at all temperatures except 45°C. This was accompanied by an increase in oil acidity.

Confirmatory evidence on the relationship between temperature and respiratory rate, carbon dioxide concentration and respiratory suppression, and the relationship of mold growth to seed respiration was provided by continuing the experiment reported in the previous section on the replicability of respiration measurements. Aeration

rates of 250, 500, 750, 1000, 1500, and 2000 ml were imposed on the six lots of soybeans maintained at a common moisture value of 18.4% which had previously been respiring for 15 days at a common aeration rate of 2000 ml per day. Following the establishment of equilibrium conditions at 30°C, a temperature of 40°C was employed (twenty-second day) until the thirty-first day when the bath temperature was raised to 50°C and held at this value until the thirty-ninth day, when it was lowered to 30°C. Graphical data for this experiment, relating differential aeration to respiratory behavior at various temperatures applied in sequence to the same lot of seeds, are given in Figure 4.

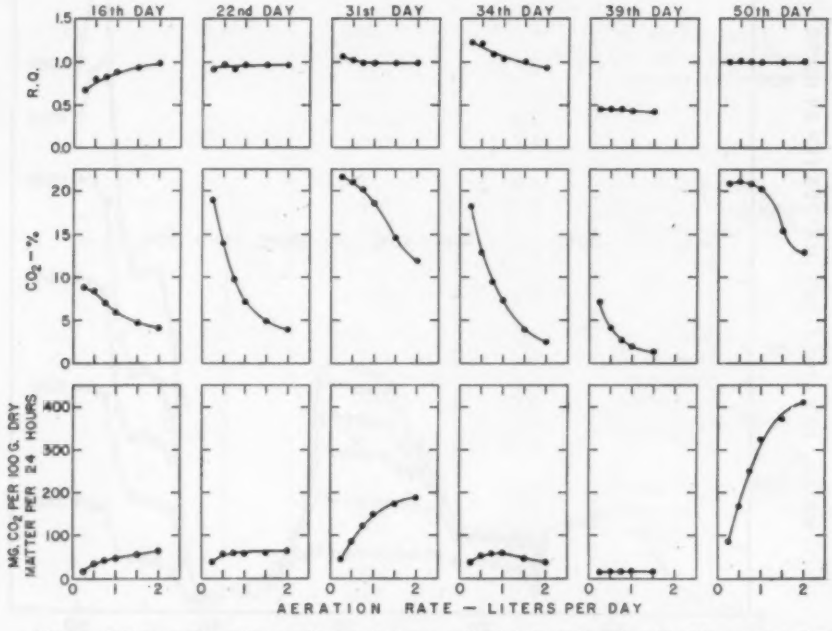

Fig. 5. Influence of aeration on the respiratory quotient, interseed carbon dioxide concentration, and carbon dioxide production of Illini soybeans respiring at 18.4% moisture. Data are for days selected at intervals throughout the trial.

Figure 5 presents the relationship found between aeration and respiratory quotient, interseed carbon dioxide concentration, and carbon dioxide production for selected days throughout the course of the trial shown in Figure 4. For temperatures up to and including 40°C, this experiment yielded similar results to the previous one in which fresh portions of seed were used for each temperature. However, the respiratory characteristics at 50°C, as well as the subsequent respiratory behavior at 30°C, merit special attention.

Following the thirty-first day of the trial (Figure 4) when the seeds had reached virtual respiratory equilibrium at 40°C and the temperature of 50°C was imposed, a drastic decrease in the respiratory rate,

particularly at the higher aeration values, occurred and continued until the temperature was changed to 30°C on the thirty-ninth day. The data for the thirty-fourth day (Figure 5) represent an intermediate point in this interval of respiratory inhibition.

At the end of eight days at 50°C (thirty-ninth day), the respiration had fallen to extremely low values and the respiratory quotients had slowly decreased to between 0.42 and 0.45. The high temperature apparently resulted in an inactivation of all biological respiration. The slow continuing carbon dioxide production, as well as the very low R.Q. values for this period, was probably due to thermal decomposition of the labile organic materials of the seeds. Within a few days after the trials at 50°C were begun, the effluent air from the respirometers had taken on a strong, sour odor, characteristic of heating grain.

On the thirty-eighth day, the respirometer receiving 2,000 ml of aeration was removed and the seeds were examined for viability and for microflora. Visually, the beans ranged in color from dark tan to brown, characteristic of bin-burned seeds. A few loose, blackened shreds of mold mycelia were visible. After surface sterilization and culturing on agar, 56% of the seeds developed mold growth, all of the colonies except one being of the *Aspergillus* species. No bacteria grew from any of the seeds examined. The germination of the sample was zero. It was apparent that although the seeds were dead, a considerable number of mold spores were able to survive at 50°C for eight days.

Following the thirty-ninth day when the bath temperature was lowered to 30°C, a further decrease in carbon dioxide production in the presumed absence of biological respiration would seem to add some support to the suggestion that thermal decomposition was involved at this stage. On the forty-first day, however, a sharp increase in the R.Q. appeared, and on the forty-second day a very rapid rise in carbon dioxide production commenced (Figure 4). By the forty-fourth day the carbon dioxide production from the highest aeration had reached a point far in excess of that at 40°C where both seed and mold respiration were undoubtedly involved. Interseed carbon dioxide values of from 19.6% to 21.7% at this time, with corresponding oxygen values in the order of 0.1%, suggested that the plateau of the respiration curves shown on the forty-fifth and forty-sixth days was due to insufficient aeration. Half of all the samples was removed at this point, thus doubling the effective aeration. Examination of the seeds removed showed that fresh, heavy mold growth was under way, the sample from the highest aeration now showing that 86% of the seeds were infected with *Aspergillus*. The effect of the increased aeration

was immediate, the carbon dioxide production practically doubling in the two succeeding days. Surprisingly, however, the respiration of the samples aerated at 1,500 ml and 2,000 ml abruptly reversed this sharply increasing trend on the forty-eighth day when the carbon dioxide concentration reached 21% with practically complete exhaustion of the oxygen. It may be that this reversal in respiration is due to an accumulation of metabolic end products toxic to molds, thus inhibiting the growth and respiration of these microorganisms.

The R.Q. values for all aerations on the final day of the trial (Figure 5, fiftieth day) were essentially 1.0 when the carbon dioxide concentration of the effluent gases from the lowest aerations was in the order of 21%. This would indicate that the R.Q. of purely mold respiration is unaffected by high carbon dioxide concentration. The anomalies in R.Q. of the samples under restricted aeration at temperatures below 50°C before this treatment was applied (sixteenth to thirty-first day) must be associated with gas exchange by the seed.

Discussion

The experimental method employed in this study, which provides for simultaneous measurement of oxygen and carbon dioxide under conditions of continuous and controlled aeration, has emphasized the importance of maintaining the interseed carbon dioxide concentration below an inhibitory level and of carrying out the trials over a much longer period of time than was heretofore employed, if the potential respiratory activities under any given set of conditions are to be revealed. The replicability of the test, as indicated by coefficients of variability in the order of 5% to 6% for respiratory activities of from 65 to 80 mg carbon dioxide per 100 g dry matter, is very satisfactory for physiological studies of this nature.

The marked similarity of respiratory increases in soybeans containing high storage moistures to microbiological growth curves is a striking feature of the present study. The increase in respiration with time appears to be directly associated with mold proliferation.

The direct relationship between aeration, mold growth, and increased oil acidity confirms similar observations by Swanson (1934) and by Pap (as presented by Rozsa, 1935) for wheat. That the fat splitting which occurred when the seeds were aerated was due principally to lipase activity by the molds is indicated by the low oil acidity values of the samples ventilated with nitrogen. Under nitrogen, no mold growth occurred and only a small increase in oil acidity was observed with time as compared with the marked increases in aerated samples. The seeds under nitrogen respired, on the average, at constant rates of about 3% of the maximum shown by similar samples

receiving optimum aeration. The very high R.Q. values of 3 to 23 for the respiration under nitrogen containing a trace of oxygen indicate its anaerobic nature.

The influence of aeration, time, and temperature on the respiration of damp soybeans, where the same sample was used throughout, suggests that viable soybeans are more resistant to mold attack than heated, nonviable seeds. It was notable that following the treatment at 50°C, the subsequent respiration at 30°C, due to molds, was two and one half times the maximum combined respiration of the molds and the seeds at a more favorable temperature (40°C) before the higher inhibitory temperature was applied. Thermal killing of the seed and the deterioration due to heat apparently favored the growth of a saprophytic mold population with consequent increases in respiratory potential. Under practical conditions, it would appear likely that seeds damaged by heat or other means would provide a more favorable medium for mold growth than would sound seeds, with correspondingly greater tendencies to heat. It would be expected that any condition prevailing during the history of a seed before or after harvesting which promotes nutrient availability for mold growth and favors an increase in the mold population would intensify subsequent storage hazards. Thus, grain damaged by mechanical injury to the seed coats, by freezing, weathering, or by remaining moist for prolonged intervals before and after harvest would require more critical storage supervision than unbroken, well-matured seeds.

In the trials with viable seeds, the respiratory quotients were invariably 1.0 when maximum respiratory activity was reached. When the aeration was decreased so that the interseed carbon dioxide concentrations exceeded 12 to 14%, the extent of mold growth was inhibited, the respiratory rate and temperature coefficients were sharply lowered, and the respiratory quotients were depressed to as low as 0.85. On the other hand, when the seeds were treated at 50°C, a temperature which allowed the survival of mold spores, but killed the seed, subsequent respiration at 30°C showed respiratory quotients of 1.0 under interseed carbon dioxide concentrations as high as 21%. These results suggest that the innate seed respiration manifests a respiratory quotient less than 0.85, whereas the respiration of the molds (principally *Aspergillus* species) has a respiratory quotient of 1.0. The nature of seed and mold respiration is being made the subject of further inquiry.

Summary

The influence of time, aeration, and temperature on the respiration of soybean seed containing approximately 18.5% moisture was investi-

gated by a technique which provided for the simultaneous measurement of oxygen consumption and carbon dioxide production under conditions of continuous and controlled aeration.

Respiration-time studies conducted up to three weeks yielded curves similar in form to microbiological population growth curves.

Aeration rate markedly influenced respiratory activity when insufficient to maintain concentrations of carbon dioxide in the interseed air below approximately 12 to 14%. Respiration under nitrogen remained constant with time at a rate of approximately 3% of that under optimum aeration.

Temperature studies conducted at 25° to 45°C in increments of 5°C showed maximum respiratory activity at 40°C. Inhibition of respiration was very marked at 50°C. The temperature coefficient increased with aeration until optimum aeration was reached.

Thermal killing of the seeds at 50°C, which permitted survival of mold spores, markedly increased the subsequent respiration at 30°C above that of the combined viable seed and indigenous mold respiration at the optimum temperature of 40°C.

Respiratory quotients of 1.0 were exhibited under optimum aeration conditions and appeared to be characteristic of mold (*Aspergillus* species) respiration. Indirect evidence indicated that the respiratory quotient of dormant soybean seed respiration is below 0.85.

Mold proliferation, as indicated by visual condition of the seed, was positively correlated with respiratory activity (and thus with degree of aeration up to the optimum) and with increases in temperature to 40°C.

Acid value of the oil extracted from soybeans at the end of respiration trials increased with aeration, with respiratory activity, and with mold proliferation. Only slight increases with time appeared in samples respiring under nitrogen.

Acknowledgment

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Literature Cited

Bailey, C. H.
1921 Respiration of shelled corn. Minn. Agr. Expt. Sta. Tech. Bull. 3.
1940 Respiration of cereal grains and flaxseed. Plant Physiol. 15: 257–274.
—— and Gurjar, A. M.
1918 Respiration of stored wheat. J. Agr. Research 12: 685–713.
Best, C. H., and Taylor, N. B.
1939 The Physiological Basis of Medical Practice (Second Ed.). Williams and Wilkins Co., Baltimore, Md.
Coleman, D. A., Rothgeb, B. E., and Fellows, H. C.
1928 Respiration of sorghum grains. U.S.D.A. Tech. Bull. 100.

Howe, R. W.
1943 An investigation of the changes in a bin of stored wheat infested by insects. Bull. Entomol. Research **34**: 145–158.
Larmour, R. K., Clayton, J. S., and Wrenshall, C. L.
1935 A study of the respiration and heating of damp wheat. Can. J. Research **12**: 627–645.
Leach, W.
1932 Further experimental methods in connexion with the use of the katharometer for the measurement of respiration. Ann. Botany **46**: 583–596.
Mangin, L.
1896 Sur la vegetation dans une atmosphere vicee par la respiration. Compt. rend. acad. sci. **122**: 747–749.
Milner, M., and Geddes, W. F.
1945 Grain storage studies. I. Influence of localized heating of soybeans on interseed air movements. Cereal Chem. **22**: 477–483.
——, Warshowsky, B., Tervet, I. W., and Geddes, W. F.
1943 The viability, chemical composition and internal microflora of frost damaged soybeans. Oil and Soap **20**: 265–268.
Oxley, T. A.
1944 The properties of grain in bulk. III. The thermal conductivity of wheat, maize and oats. J. Soc. Chem. Ind. **63**: 53–55.
Peters, J. P., and Van Slyke, D. D.
1932 Quantitative Clinical Chemistry. Volume II. Methods. Williams and Wilkins Co., Baltimore, Md.
Ramstad, P. E., and Geddes, W. F.
1942 The respiration and storage behavior of soybeans. Minn. Agr. Expt. Sta. Tech. Bull. 156.
Rozsa, T. A.
1935 Drinking habits of the wheat berry. Northwestern Miller, Production Annual **184** (2): 73–74.
Shirk, H. G., and Appleman, C. O.
1940 Oxygen respiration in wheat grain in relation to freezable water. Am. J. Botany **27**: 613–619.
Spencer, H. M.
1926 Laboratory methods for maintaining constant humidity. International Critical Tables, Vol. I: 67–68. McGraw-Hill Book Company, New York.
Stiles, W., and Leach, W.
1931 On the use of the katharometer for the measurement of respiration. Ann. Botany **45**: 461–488.
Swanson, C. O.
1934 Some factors involved in damage to wheat quality. Cereal Chem. **11**: 173–199.
—— and Fenton, F. C.
1932 The quality of wheat as affected by farm storage. Kan. Agr. Expt. Sta. Tech. Bull. 33.
Taylor, D. L.
1942 Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. Am. J. Botany **29**: 721–738.
Tervet, I. W.
1945 The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopathology **35**: 3–15.
Wilson, R. E.
1921 Humidity control by means of sulphuric acid solutions, with critical compilation of vapor pressure data. Ind. Eng. Chem. **13**: 326–331.
Zeleny, L., and Coleman, D. A.
1938 Acidity in cereals and cereal products, its determination and significance. Cereal Chem. **15**: 580–595.

ACTIVITY-pH RELATIONSHIPS AND INHIBITION OF PROTEOLYTIC ENZYME ACTIVITY OF MILLED FRACTIONS OF WHEAT

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An investigation of certain relatively neglected aspects of the proteolytic enzyme activities in wheat was undertaken, not only to obtain fundamental data, but also to gather information that might be used to examine the validity of the proteolytic enzyme theory of the effect of oxidation on dough properties. The application of the data obtained to the latter problem will form the substance of a later communication.

The present study is concerned first with the activity-pH relationships that exist when protease acts on its concomitant naturally occurring substrate under conditions least likely to effect change in either the enzyme or substrate. Parallel investigations of these relationships in the presence of an added substrate, casein, were also carried out. Casein was employed after it was observed that this substrate was enzymatically hydrolyzed much more readily than gluten.

A second phase of the study embraced the search for an inhibitor of the protease action which would not influence the state of oxidation of the system, and which, at the same time, would be sufficiently powerful and of such a nature as to have a minimum effect on the substrate protein while exerting an appreciable effect on the protease. Octyl alcohol, hexylresorcinol,[1] and sodium fluoride were found to have merit in this regard. The use of ultraviolet irradiation was also examined as a means of inhibiting the enzymatic process, since radiations in the ultraviolet region are known to inactivate other proteases such as trypsin and papain (Kauffman and Urbain, 1944).

Experimental

Extraction. In order to obtain an idea of the relative values of distilled water, 1% sodium chloride and 30% glycerol as extracting media for the protease, 15 g of ether-extracted wheat germ and 2 drops of chloroform were added to 100 ml of each extractant. After 72 hours at 37°C, the mixtures were centrifuged and the supernatant fluids were filtered through No. 4 Whatman paper. Kjeldahl nitrogen determinations were carried out on each of the extracts. Ten ml portions of

[1] After this work had been completed, a paper by Horwitt (1944) appeared in which it was shown that hexylresorcinol is an inhibitor of both trypsin and chymotrypsin.

extract were combined with 1 ml of a solution of neutralized cysteine hydrochloride (15 mg), and titrations for amino groups, performed as described below, were carried out before and after a 23-hour period during which the temperature of the mixtures was held at 37°C. The results of this experiment are given in Table I, which shows that, while

TABLE I

COMPARISON OF THE PROTEOLYTIC ACTIVITY OF WATER, GLYCEROL, AND SALINE EXTRACTS OF WHEAT GERM

	Protease activity ml 0.1 *N* HCl	Total nitrogen per ml of extract	Protease activity per mg N ml 0.1 *N* HCl
		mg	*ml*
Water	2.00	5.20	.038
30% glycerol	0.80	6.10	.013
1% sodium chloride	1.40	5.14	.027

the 30% glycerol extracted the most nitrogen, it demonstrated the least proteolytic autolysis. The water extract not only underwent the greatest autolysis, but it showed the greatest effect per mg nitrogen. That the proteolysis is almost completely enzymatic was found by the use of boiled extracts which gave negligible increases in amino groups.

The extraction procedure finally adopted was the following: The quantity of material extracted was 30 g for germ or bran, 67 g for patent, clear, or low grade flours, and 57 g for shorts and whole wheat flour. The sample was added to 200 ml of distilled water and stirred for 1 hour at room temperature. The suspension was centrifuged and the supernatant extract was decanted and used as the source of enzyme. The proportion of the amount of a wheat product to the extractant was rather arbitrarily chosen so that an excess of the wheat product was used.

Titration of Amino and Carboxyl Groups. Amino groups were titrated by the Linderstrøm-Lang (1927) acetone method. Ten ml aliquots were titrated with 0.1 *N* alcoholic hydrochloric acid until most of the acid required was added; then 100 ml of acetone containing 0.5 mg % naphthyl red (Grübler) was run in, and the titration was finished with the alcoholic hydrochloric acid, matching the color of the end point to that of a control sample.

The carboxyl groups were titrated by the Willstätter and Waldschmidt-Leitz (1921) alcohol method. Ten ml aliquots were titrated with 0.1 *N* alcoholic potassium hydroxide solution after 100 ml of 95% alcohol and 10 drops of 1% alcoholic thymolphthalein were added. The end-point color was matched to that of a control sample.

Reaction Mixtures. Ten ml of extract was combined with 10 ml of M/7 sodium acetate, 5 ml of cysteine hydrochloride solution containing

37.5 mg, 5 ml of distilled water, and either glacial acetic acid, 1.0 *N* acetic acid, or 1.0 *N* sodium hydroxide to bring the pH to its proper value. Distilled water was then added to make a total volume of 35 ml. A glass electrode was used in adjusting the pH. An aliquot of 10 ml was used for amino titration at once, and another equal aliquot, with a drop or two of toluene, was introduced into a flask which was stoppered and placed in a thermostat at 37°C for the digestion period and then titrated for amino groups.

In experiments designed to demonstrate the effect of added casein, the 5 ml of water used in the preceding reaction mixture was replaced by 5 ml of 6% casein solution. This was prepared by dissolving 6 g of casein (Hammarsten) in 72 ml of 0.1 *N* sodium hydroxide and diluting to 100 ml with distilled water. The possibility that the casein contained milk protease could not influence the present results since this enzyme has practically no action in the pH range employed (Warner and Polis, 1945).

In accordance with the original observation of Hullett and Stern (1941) and its confirmation by Smith and Geddes (1942), reaction mixtures containing germ extract, at pH values above 5.0, developed a violet color after standing 12 hours or longer. This color interfered with the end point in the amino titrations. The pigment formation seems to involve an enzymatic oxidation, as suggested by Hullett and Stern (1941), since it was prevented by boiling the extract or replacing the air over the liquid by nitrogen. Toluene appeared to inhibit the action.

Digestion Periods and Hydrogen Ion Concentration. In order to determine a suitable digestion period for the proteolysis effected by each milled wheat fraction, a preliminary study of the influence of digestion time on hydrogen ion concentration was carried out. The pH after various intervals was measured in a reaction mixture consisting of 15 ml of germ extract, 10 ml of M/7 sodium acetate, 5 ml of cysteine hydrochloride (37.5 mg), and 5 ml of distilled water. The air in the flask was replaced by nitrogen to prevent pigment formation. A parallel experiment was set up in which the cysteine hydrochloride was replaced by water. The initial pH was 6.47, and this value was chosen since it was the highest used in the determination of activity-pH relationships, and the greatest change in pH occurred at this value. Hence the experiment represented the extreme case. The results are given by the curves in Figure 1. Since 9 hours was the time just before the sudden increase in hydrogen ion concentration, in the presence of cysteine, this interval was chosen as the digestion period for germ extract; and it was also found to be the appropriate period for digestions involving whole wheat and shorts. Similarly, a 24 hour digestion

period was found to be best for the bran, patent, clear, and low grade flour experiments.

In order to elucidate the reason for the drop in pH at the higher pH values, titrations of both amino and carboxyl groups were performed, parallel with determinations of hydrogen ion concentration, on boiled extracts of germ before and after a 24 hour period at 37°C, with and without M/7 acetate buffer. The results are summarized in Table II and will be discussed later.

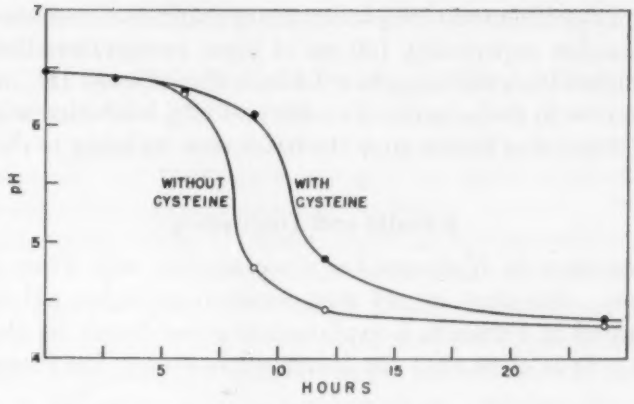

Fig. 1. Effect of digestion time at 37°C on the pH of buffered germ extract.

In the final experiments, a check-up of the hydrogen ion concentration at the end of the digestion period was carried out in each case. For this purpose, the 15 ml of reaction mixture remaining after the two 10-ml portions had been removed (one for immediate titration and the other for digestion) was placed in a flask with a drop or two of toluene. The flask was stoppered and placed in the thermostat.

Inhibition of Protease. All inhibition studies were carried out on the protease of germ extract. When *n*-octyl alcohol was employed as the inhibitor, it was added to the total 35 ml of reaction mixture, but with sodium fluoride and hexylresorcinol either a 1 or 2% solution was used to replace a portion of the water employed in making up the final volume to 35 ml. The experiments employing the germ extract, buffer, and cysteine, in the proportions used for the determinations in the activity-pH work, were carried out for 9 hours at 37°C at the optimum pH.

Experiments designed to show the effect of ultraviolet radiation on the protease were performed as follows: In one case 15 g of dry ether-extracted germ was spread out in a thin layer 7 inches below a Hanovia ultraviolet lamp. All of the germ was in the field of the radiation.

After 24 hours of irradiation the material was extracted, and its protease activity determined at pH 5.0, with and without casein, as usual. Comparison with the protease action of a similar sample subjected to identical treatment, with the exception of the irradiation, showed that the ultraviolet radiation of the dry sample had no demonstrable effect.

In another experiment 50 ml of germ extract was irradiated in a 6.5-inch evaporating dish for 18 hours, 7 inches from the Hanovia lamp. The water evaporated completely, and the residue was taken up in 50 ml of distilled water. Measurement of the protease action in the presence of casein showed 28% less activity than a nonirradiated control. In still another experiment 150 ml of germ extract, irradiated for 3 hours, 9 inches from the lamp, in a 9.5-inch dish, showed 16% inhibition of the protease in the presence of casein and 12% inhibition without the casein. Water was added after the irradiation to bring to the original volume.

Results and Discussion

The Increase in Hydrogen Ion Concentration with Time at Higher pH Values. The drop in pH that occurred at higher pH values, as demonstrated in Figure 1, is explained to some degree by the data in Table II. It is clear that the increased hydrogen ion concentration

TABLE II
CHANGE IN PH AFTER 24 HOURS ASSOCIATED WITH NONENZYMATIC AUTOLYSIS OF WHEAT GERM EXTRACT AT 37°C

Composition of reaction mixture [1]	pH		Increase in	
	Initial	Final	NH_3	COOH
			Ml 0.1 *N* HCl	Ml 0.1 *N* KOH
10 ml boiled germ extract, 25 ml water	6.57	4.01	0.10	2.70
10 ml boiled germ extract, 10 ml M/7 acetate buffer, 15 ml water	6.60	4.97	0.00	3.65

[1] Saturated with toluene.

results from the nonenzymatic liberation of carboxyl groups. The greater increase in carboxyl, when buffer is present, is the result of the higher pH that is maintained. This was confirmed in a separate experiment in which buffers ranging from pH 4.0 to 6.5 were employed. The liberation of carboxyl groups cannot be accounted for by hydrolysis of peptide linkages, since this would be accompanied by the simultaneous liberation of amino groups. It might be that nonenzymatic hydrolysis of ester linkages was responsible. The ether extraction of the germ was not exhaustive, and, since ether does not remove all of

the lipid (Sullivan and Near, 1933; Sullivan and Bailey, 1936), some lipid material was undoubtedly still present.

From the foregoing it is clear that the increase in amino groups is more reliable as a measure of proteolysis than the increase in carboxyl groups which, by means of the formol titration, has been the prevalent practice. Furthermore the acetone titration is a little simpler to carry out than the formol titration. Balls and Hale (1935) employed the alcohol titration as a means of measuring proteolysis. The alcohol titration with alkali is particularly well suited to the determination of carboxyl groups, and the acetone titration with acid to the measurement of amino groups.

Samuel (1934) reported that formol titrations indicated no increase in amino acid when water extracts of flour were allowed to stand. This inference was occasioned, no doubt, by the fact that the extracts did not stand long enough, and both temperature and hydrogen ion concentration were not optimal.

The pH Optima. The pH optimum found was around 5.0 for whole wheat, germ, clear, and patent flours with and without casein. Although the optimum found was about 5.0 for shorts, low grade flour, and bran with casein, it was closer to 4.5 without the added substrate (Figure 2). The reason for the shift in the pH optimum in the absence of casein in the latter instances is not clear. Apparently the components of the outer layers of the grain which are contained in bran, shorts, and low grade flour, but which are relatively absent in germ, clear, and patent flours, and which are present in lower concentrations in whole wheat flour, are responsible for the difference.

The hydrogen ion concentration remained constant during digestion in all of these cases except at the two or three highest pH values, and even at these values it was constant when extracts of whole wheat flour were used. The extent of the pH changes that were encountered under the conditions employed with extracts of each milling fraction may be seen from Figure 2.

The activity-pH curves should not be used as a basis for comparison of the quantitative protease activities of the various wheat products, since these products were stored for different periods and some loss in active enzyme undoubtedly took place. The greater activity of the whole wheat flour is due to the fact that the wheat was not ground until ready for use; the deterioration in the whole grain occurs at a slower rate than in the milled fractions. Furthermore, it is not certain that the rates of hydrolysis were linear throughout the entire 9 and 24 hour digestion periods, and linearity would, of course, be a prerequisite to any such comparison.

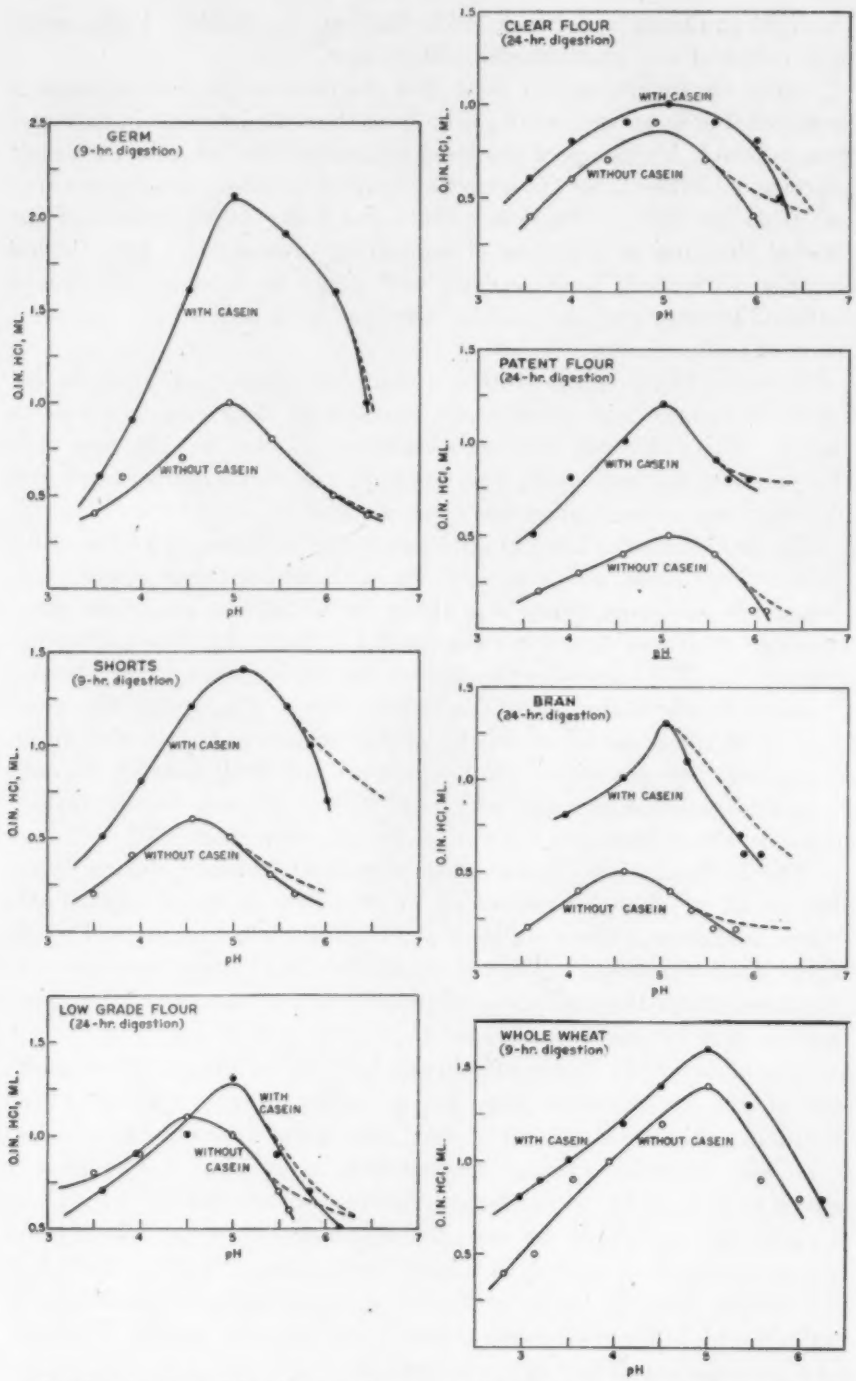

Fig. 2. Protease activity-pH curves for extracts of germ, shorts, low grade flour, clear flour, patent flour, bran, and whole wheat. Broken line indicates initial pH; solid line, average pH.

In a group of papers on the proteolytic enzymes of sprouted wheat, Mounfield (1936, 1936a) observed a pH optimum of 4.1 when edestin was employed as substrate, 5.1 when gelatin was used, and 6.0 when gluten dispersed in acetic acid was added. In the second paper (1936a) it was shown that activation of the protease by cyanide effected a shift in the pH optimum from 4.1 to 4.8 with edestin as substrate. Mounfield made the interesting observation that both citrate and chloride ions inhibit the protease and that citrate, phthalate, and glycine buffers, the latter two because of associated chloride ions, permit little enzyme activity. Acetate buffers were found to be suitable, however. Our own preliminary experiments with citrate, phthalate, and acetate buffers are in accord with these findings.

As part of a study on the histochemical distribution of protease in wheat, Pett (1935) observed a pH optimum at 5.0 when edestin was employed as substrate. Balls and Hale (1935) measured protease activity in suspensions of flour in water to which hydrochloric acid had been added to adjust the pH to various values. No buffer was employed, it being assumed apparently that the buffer capacity of the flour would be sufficient to prevent a change in hydrogen ion concentration. Maximum activity was reported at pH 4.5 for "white" flour, and two maxima, one at pH 5.0 and another at 3.3, were given for whole wheat flour. During the course of our investigation it was found that a buffer is required, and extracts of whole wheat flour exhibited a single maximum in the region of pH 5.0.

It is a surprising fact that in none of the previous publications on wheat protease have measurements of hydrogen ion concentration been reported, after digestion, to determine whether changes had taken place. We have found that it is necessary to check the hydrogen ion concentration after, as well as before, digestion even when buffers are employed. This point should be borne in mind in the interpretation of previous work.

It is of interest to review the papers of Blagoveschenski and coworkers (1933, 1935, 1937) since they present some points of view that are at variance with our conclusions and also with those of others. In the three papers of Blagoveschenski *et al.* the conclusion was drawn in each case that enzymatic proteolysis in wheat flour involved only a disaggregation of protein without the liberation of free amino groups. In the second of the papers, Blagoveschenski and Yurgenson (1935) reported that, using gliadin, globulin, and glutenin fractions of flour, and a water extract of flour as substrates, with a water extract of flour as the source of enzyme, no increase in amino-nitrogen by enzyme action was found except when the water-soluble fraction of the flour was used as substrate. They state, "Increases occur in both experimental and

control solutions but the differences between the experiments and their corresponding controls are significant only in the case of the water-soluble fraction, and here only at the more acid reactions; at pH 8.5 where the maximum solution of proteins occurs, the changes in amino-nitrogen are negligible. It is clear, therefore, that except at the acid reactions (where some hydrolysis of the dissolved proteins must be assumed), the increased solubility of the proteins which has been shown to occur under the influence of the flour enzymes is not accompanied by true proteolysis." That there is an alternative interpretation of these data is emphasized by the subsequent work of Mounfield (1936, 1936a), Pett (1935), and the present authors, all of whom have independently demonstrated the enzymatic liberation of amino groups on the acid side of neutrality. The water extract of flour used as the source of enzyme by the Russian group also contains protein that acts as a substrate. This protein, rather than the gliadin, globulin, or glutenin fractions added, is most likely preferentially hydrolyzed by the protease, in accord with the observations of Mounfield (1938). We found that adding gluten, colloidally dispersed in dilute alkali and then neutralized, to a water extract of flour produces only a small additional enzymatic proteolysis. Hence, it is not surprising that negligible increases of amino-nitrogen were observed by the Russian investigators when the gluten fractions were added as "substrate." On the other hand, the appreciable peptide scission found in water extracts of flour is to be expected since they certainly contain both enzyme and substrate, and the substrate is in a form rather easily attacked by the enzyme. Blagoveschenski's consideration that the "enzymatic" disaggregation is optimum at pH 8.5, because at this hydrogen ion concentration there is a maximum increase in soluble nitrogen, is also open to question. A pH of 8.5 is out of the range of the hydrogen ion concentrations occurring in wheat, and the effects observed *in vitro* at this pH are probably not enzymatic in character. Subsequent results from other laboratories have, in fact, established the requirement of an acid condition for wheat protease action, and Mounfield (1936) showed that at pH 8.0 a rapid destruction of the wheat enzyme occurs.

Inhibition of Protease. The degree of inhibition of the protease of germ by *n*-octyl alcohol, hexylresorcinol, and sodium fluoride is shown in Figure 3. The presence of casein in the reaction mixture depressed the maximum inhibition attained with *n*-octyl alcohol from 57 to 42% and with sodium fluoride from 57 to 29%. However, hexylresorcinol inhibition was influenced but little when casein was employed, since the maximum was 60% without, and 65% with, casein. The effect of ultraviolet radiation was a small inhibition when extracts were treated, and none when the dry germ was subjected to the irradiation.

The observation of Freilich and Frey (1943) [2] and of Shen and Geddes (1942) that formol titrations of dough increase in the presence of octyl alcohol is interesting with reference to our findings. Apparently octyl alcohol exhibits a multiple action. It blocks fermentation and simultaneously abolishes the factor, probably enzymatic, that

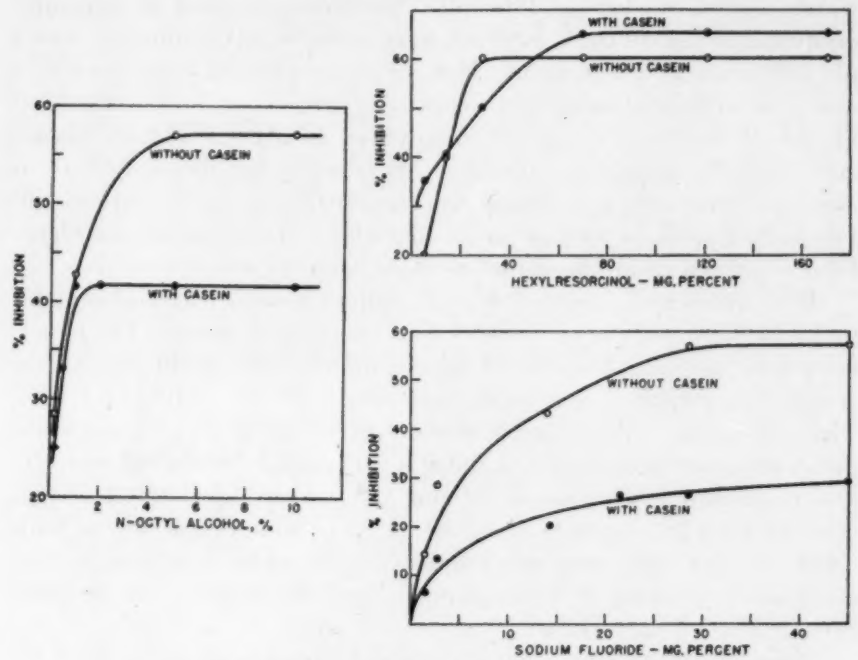

Fig. 3. Inhibition of the protease in germ extract by *n*-octyl alcohol, hexylresorcinol, and sodium fluoride.

results in a disappearance of amino-nitrogen, as Freilich and Frey, and Shen and Geddes demonstrated. It also inhibits the flour protease that produces an increase in amino groups as proved by data in this report. The increase in amino groups by proteolysis must be more than compensated by the factor causing disappearance of these groups during dough fermentation since, in the absence of octyl alcohol, a net loss in amino groups occurs. From our own results it is clear that octyl alcohol does not prevent all protease action; it has no further influence after a certain maximum inhibition is attained. Therefore, some proteolysis will proceed in the presence of octyl alcohol, while the factor that decreases amino nitrogen is apparently entirely eliminated. This combination of actions would effect a change during fermentation of the dough from a decrease in amino groups in the absence of octyl

[2] First reported at the A.A.C.C. meeting in New York, May, 1940.

alcohol to an increase in these groups in the presence of the compound. This phenomenon was what Freilich and Frey, and Shen and Geddes found.

Summary

Measurements of activity-pH relationships for the protease in water extracts of wheat and its milled fractions, acting on the naturally occurring protein in these extracts, have revealed an optimum at about pH 5.0 for whole wheat, germ, clear, and patent flours, with or without casein as additional substrate. However, extracts of shorts, bran, and low grade flour showed an optimum closer to pH 4.5 without casein, and 5.0 with the added substrate. It is necessary to use buffers to maintain constancy of hydrogen ion concentration, and the pH should be checked after, as well as before, digestion. These precautions have been neglected in much of the previous work on wheat protease.

Hexylresorcinol, *n*-octyl alcohol, sodium fluoride, and ultraviolet radiation inhibited the protease of water extracts of germ. The hexylresorcinol in concentrations of approximately 70 and 40 mg %, respectively, exhibited maximum inhibitions of 65% with, and 60% without, casein. Maximum inhibitions of 42 and 57%, with and without casein, were produced by 2 and 5% of the octyl alcohol respectively, and maximum inhibitions of 29 and 57%, with and without casein, were effected by approximately 30 mg % of sodium fluoride in both cases. Under the conditions employed, ultraviolet radiation had no effect on the protease of dry germ and a small inhibiting action on germ extract amounting to 12 to 28 %.

A nonenzymatic liberation of carboxyl groups, without an increase in free amino groups, was observed in germ extract, and its effect on hydrogen ion concentration was measured.

Acknowledgments

The authors wish to thank Dr. W. A. Feirer of the medical research division of Sharp and Dohme, Inc., for a generous gift of the crystalline hexylresorcinol used in the present study, and to express their appreciation for the valuable criticism given by Dr. Betty Sullivan of this laboratory.

Literature Cited

Balls, A. K., and Hale, W. S.
1935 Proteolysis in flours. J. Assoc. Official Agr. Chem. **18**: 135–140.
Blagoveschenski, A. V., and Sossiedov, N. I.
1933 The gluten-dissolving ferment of wheat and barley seeds. Biochem. J. **27**: 1575–1577.
—— and Yurgenson, M. P.
1935 On the changes of wheat proteins under the action of flour and yeast enzymes. Biochem. J. **29**: 805–810.
1937 On the changes of leucosin, gliadin, and glutenin under the action of flour and yeast proteinases. Cereal Chem. **14**: 103–107.

Freilich, J., and Frey, C. N.
1943 Dough oxidation and mixing studies. V. Correlation between protease activity, reducing matter, and oxidizing effects in dough. Cereal Chem. **20**: 301–310.
Horwitt, M. K.
1944 Reactions of trypsin and chymotrypsin with heparin, trypsin inhibitor, and hexylresorcinol. J. Biol. Chem. **156**: 427–432.
Hullett, E. W., and Stern, R.
1941 Biological elimination of glutathione from wheat germ and flours used in bread making. Cereal Chem. **18**: 561–572.
Kauffman, F. L., and Urbain, W. M.
1944 The photochemical inactivation of trypsin and papain solutions in the ultraviolet region. J. Am. Chem. Soc. **66**: 1250–1253.
Linderstrøm-Lang, K.
1927 Volumetric determination of amino nitrogen. Compt. rend. Lab. Carlsberg **17** (4): 1–17. Titrimetrische Bestimmung von Aminostickstoff. Z. physiol. Chem. **173**: 32–50 (1928).
Mounfield, J. D.
1936 The proteolytic enzymes of sprouted wheat. Biochem. J. **30**: 549–557.
1936a The proteolytic enzymes of sprouted wheat. II. Biochem. J. **30**: 1778–1786.
1938 Proteolytic enzymes of sprouted wheat. III. Biochem. J. **32**: 1675–1684.
Pett, L. B.
1935 Studies on the distribution of enzymes in dormant and germinating wheat seeds. Biochem. J. **29**: 1898–1904.
Samuel, L. W.
1934 The determination of amino-acids in wheat flour. Biochem. J. **28**: 272–282.
Shen, T., and Geddes, W. F.
1942 Protease activity and reducing matter content of wheat flour doughs in relation to baking behavior. Cereal Chem. **19**: 609–631.
Smith, D. E., and Geddes, W. F.
1942 Yeast fermentation and potassium bromate as factors influencing the harmful effects of wheat germ on baking quality. Cereal Chem. **19**: 785–804.
Sullivan, B., and Bailey, C. H.
1936 The lipids of the wheat embryo. I. Fatty acids. J. Am. Chem. Soc. **58**: 383–390.
—— and Near, C.
1933 Change of crude lipides of wheat on storage. Ind. Eng. Chem. **25**: 100–103.
Warner, R. C., and Polis, E.
1945 On the presence of a proteolytic enzyme in casein. J. Am. Chem. Soc. **67**: 529–532.
Willstätter, R., and Waldschmidt-Leitz, E.
1921 Alkalimetrische Bestimmung von Aminosäuren und Peptiden. Ber. **54B**: 2988–2993.

GLUTAMIC ACID CONTENT OF FEED MATERIALS

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The shortage of animal proteins and the need for protein evaluation of plant feedstuffs have made the advancement of the knowledge of the amino acid content of proteins imperative. Microbiological methods of analysis for a number of amino acids have become available. The amino acid which occurs most abundantly in grain proteins is glutamic acid. Analytical methods for glutamic acid by chemical procedures are tedious. The whole procedure requires several months to carry through to completion, is very laborious, and demands analytical skill of high order (Kibrick, 1944). Several applications of microbiological analysis to glutamic acid determinations have been reported (Dunn, Camien, Rockland, Shankman, and Goldberg, 1944; Lewis and Olcott, 1945; Lyman, Kuiken, Blotter, and Hale, 1945).

However, difficulties are encountered in the microbiological assay. Under certain conditions standard curves for glutamic acid possessed sigmoidal shape. Such sigmoidal-shaped curves were observed when the asparagine in the medium (Baumgarten, Stone, and Boruff, 1945) was replaced by aspartic acid. Smooth curves were always obtained when the medium contained asparagine. The indiscriminate use of aspartic acid in place of asparagine must be avoided in media designed for glutamic acid assays.

Glutamic acid values determined microbiologically are highly reproducible, but discrepancies were noticed which made their absolute accuracy doubtful. Previous investigators employed *Lactobacillus arabinosus* in glutamic acid assays, in spite of the report of Dunn *et al.* (1944) that *Lactobacillus arabinosus* responded to *dl*-glutamic acid to an extent far above that accounted for by its *l* (+)-glutamic acid content. Consequently *Lactobacillus arabinosus* should be considered unsuitable as an assay organism for *l* (+)-glutamic acid if the presence of *d* (−)-glutamic acid might be suspected.

In the present study, the response of five microorganisms to *l*(+), *d*(−), and *dl*-glutamic acid was investigated. *Streptococcus faecalis* R. and *Leuconostoc mesenteroides* did not utilize *d*(−)-glutamic acid, whereas *Lactobacillus arabinosus*, *Lactobacillus acidophilus*, and *Lactobacillus casei* did respond to it. Stokes and Gunness (1945) reported that both forms of aspartic acid were available to *Lactobacillus delbruckii*. Our observations corroborate these findings that micro-

organisms, similar to animals, do not necessarily require the natural forms of amino acids.

A large number of protein-containing feed materials was assayed for glutamic acid with special emphasis directed towards the possible occurrence of *d*(−)-glutamic acid in these products.

Materials and Methods

The microbiological technic and preparation of samples have been described in detail by Baumgarten *et al.* (1945). The same medium was employed in the present study with the exceptions that the concentrations of alanine, asparagine, and serine were increased to 4 mg per tube for *Lactobacillus acidophilus* and that it contained 0.5 mg of xanthine per tube. Assays with *Lactobacillus arabinosus*, *Leuconostoc mesenteroides*, and *Streptococcus faecalis* R. were incubated at 30°C, while the ones with *Lactobacillus acidophilus* and *Lactobacillus casei* were incubated at 37°C. Analyses carried out with *Lactobacillus arabinosus*, *Lactobacillus acidophilus*, and *Lactobacillus casei* were evaluated by titration of the lactic acid formed. *Streptococcus faecalis* R. and *Leuconostoc mesenteroides* assays were determined by measuring the turbidity produced in the assay tubes, since insufficient amounts of lactic acid for titration were produced on the present medium.

The *l*(+)-glutamic acid was obtained from Merck & Company. The *dl*-glutamic acid monohydrate was supplied by the Amino Acid Manufacturers of the University of California at Los Angeles. The *d*(−)-glutamic acid was prepared according to the procedure of E. Fischer (1899).

The organisms employed were *Lactobacillus arabinosus*, *Lactobacillus acidophilus* (No. 4357 ATCC), *Lactobacillus casei*, *Leuconostoc mesenteroides* (No. 8042 ATCC), and *Streptococcus faecalis* R. (No. 8043 ATCC formerly known as *Streptococcus lactis* R.—see Gunsalus, Niven, and Sherman, 1944).

Results and Discussion

The influence of aspartic acid and asparagine on standard curves of glutamic acid is demonstrated in Figure 1 using *Lactobacillus arabinosus*. The sigmoidal-shaped curve observed when aspartic acid replaced asparagine in the medium is unsuitable for assay work. The same relationship was noted with the four other microorganisms.

Figures 2, 3, 4, and 5 demonstrate the response of the assay organisms to the optical forms of glutamic acid. The behavior of *Leuconostoc mesenteroides* and *Streptococcus faecalis* R. was identical and the curves for the former were omitted.

The effect of asparagine and aspartic acid on the standard curves of glutamic acid is of significance. Lyman *et al.* (1945) reported that the

addition of small amounts of glutamine to a medium containing aspartic acid (Kuiken, Norman, Lyman, Hale, and Blotter, 1943) would produce a smooth standard curve. However, glutamine should be present

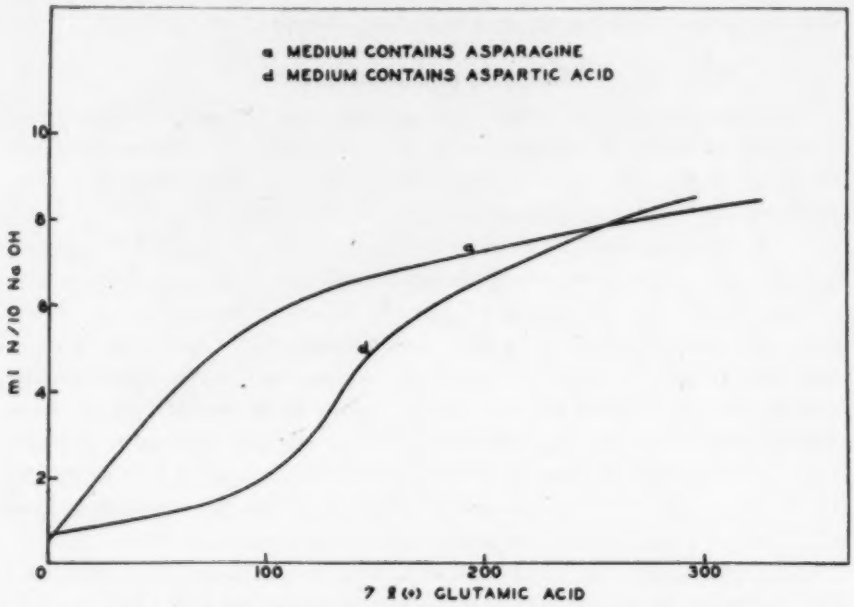

Fig. 1. Effect of asparagine and aspartic acid on standard curves for glutamic acid with *L. arabinosus*.

only in minute amounts as it possesses glutamic acid activity. During autoclaving, glutamine is converted largely to the ammonium salt of pyrrolidonecarboxylic acid which is devoid of activity. Consequently glutamine has to be added either aseptically after autoclaving or in increased amounts to compensate for the losses incurred during autoclaving, which might lead occasionally to high blanks. It is apparent that the inclusion of asparagine is simpler as it does not undergo any appreciable changes during heat sterilization. It should be pointed out that aspartic acid and asparagine can be substituted for each other (Shankman, 1943) in assays for amino acids other than glutamic acid.

It has been mentioned above that the assumption of the sole utilization of the *l*-amino acids by these microorganisms was detected invalid in several instances. Since the methods for the analysis of glutamic acid with *Lactobacillus arabinosus* did not clearly distinguish between *l*(+) and *d*(−)-glutamic acid, a more specific method was desirable.

Five microorganisms have been studied. *Leuconostoc mesenteroides* and *Streptococcus faecalis* R. could utilize only the *l*(+) form (Figure 2); the *d* form was entirely inactive and the response manifested by the *dl* form was half the one observed for the *l* form. *Lactobacillus casei*

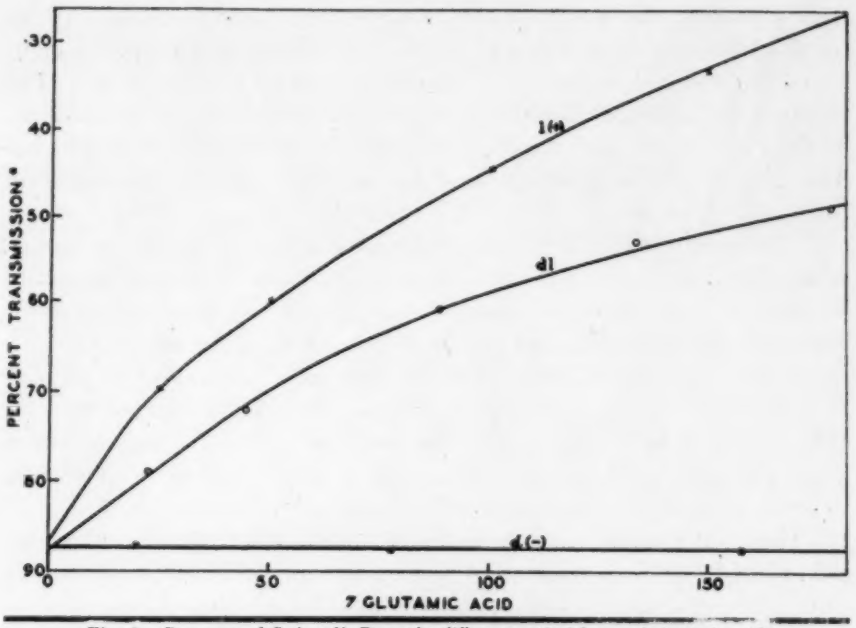

Fig. 2. Response of *S. faecalis* R. to the different optical forms of glutamic acid.

* The turbidity of the solutions was expressed in percent transmission measured in a Coleman universal spectrophotometer using square cuvettes of 13 mm diameter.

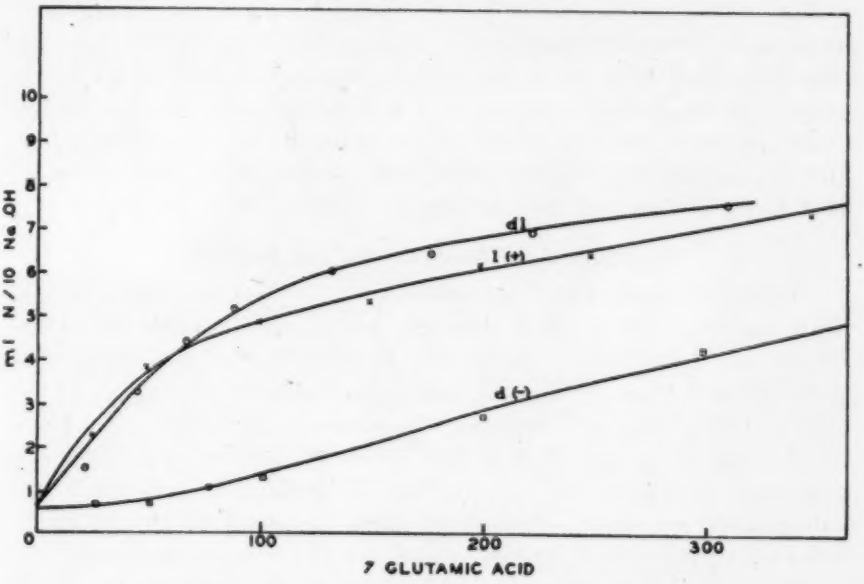

Fig. 3. Response of *L. casei* to the different optical forms of glutamic acid.

(Figure 3) apparently did not distinguish between *l*(+) and *dl*-glutamic acid while *d*(−)-glutamic acid possessed activity. The response of *Lactobacillus arabinosus* and *Lactobacillus acidophilus* (Figures 4 and 5)

to *dl*-glutamic acid was irregular. *dl*-Glutamic acid had less activity for *Lactobacillus acidophilus* than the *l*(+) form on an equal weight basis, but more than would be expected from its *l*(+) content. The reaction of *Lactobacillus arabinosus* to *dl*-glutamic acid was remarkable, as the ratio of the activity changed with the concentration employed. The activity of *l*(+)-glutamic acid was replaced by *d*(−)-glutamic acid for both organisms.

The curves with *d*(−)-glutamic acid were sigmoidal in shape, whereas the ones obtained with *l*(+) or *dl*-glutamic acid were of normal shape. It was observed visually that the tubes containing *d*(−)-glutamic acid showed a slower rate of growth than the ones with *l*(+) or *dl*-glutamic acid, but no apparent difference in growth rate seemed to exist between the *l*(+) and *dl* forms. This lag indicated that an enzyme might be present which was instrumental in the conversion of *d*(−)-glutamic acid into *l*(+)-glutamic acid and whose activity was dependent upon the concentrations of the substrate.

The results of the response to the different optical forms of glutamic acid must be taken into account when glutamic acid assays are carried out. Any one of the five organisms may be employed provided no racemization of glutamic acid had taken place. The percentage of *l*(+)-glutamic acid may be determined with *Streptococcus faecalis* R. or *Leuconostoc mesenteroides*. Determinations with *Lactobacillus casei* as an assay organism should give the total amount of glutamic acid present regardless of the optical configuration, provided a substantial amount of the *l* form is present. The total amount of glutamic acid in an unknown solution as well as the respective percentages of *l*(+) and *d*(−)-glutamic acid could conceivably be determined by employing both *Lactobacillus casei* and *Streptococcus faecalis* R.

Glutamic Acid Analysis of Feed Samples

A large number of feed materials was analyzed for their glutamic acid content. The values obtained are given in Table 1. Three organisms, *Lactobacillus arabinosus*, *Lactobacillus casei*, and *Streptococcus faecalis* R., were employed in the microbiological assay. It was observed that in every instance the values determined with *Lactobacillus arabinosus* were the highest, while the ones with *Lactobacillus casei* were intermediate and *Streptococcus faecalis* R. gave the lowest values with one single exception. These results lend weight to the belief that part of the glutamic acid is present in racemized form.

Racemization might conceivably occur during hydrolysis. *d*(−)-Glutamic acid might be present in the intact protein. This fact should be taken into consideration in microbiological assays, and the response of the organisms to the optical forms of amino acids must be kept in mind in the evaluation of the results.

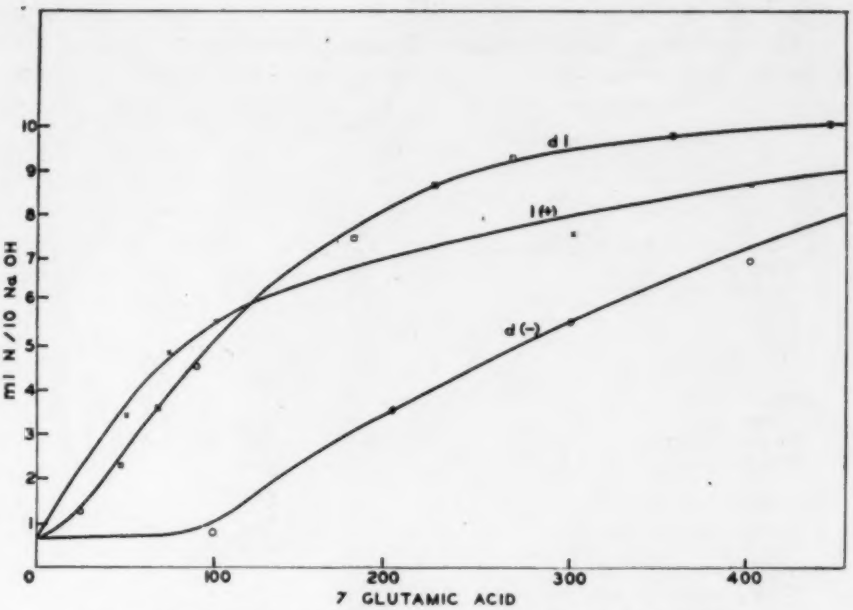

Fig. 4. Response of *L. arabinosus* to the different optical forms of glutamic acid.

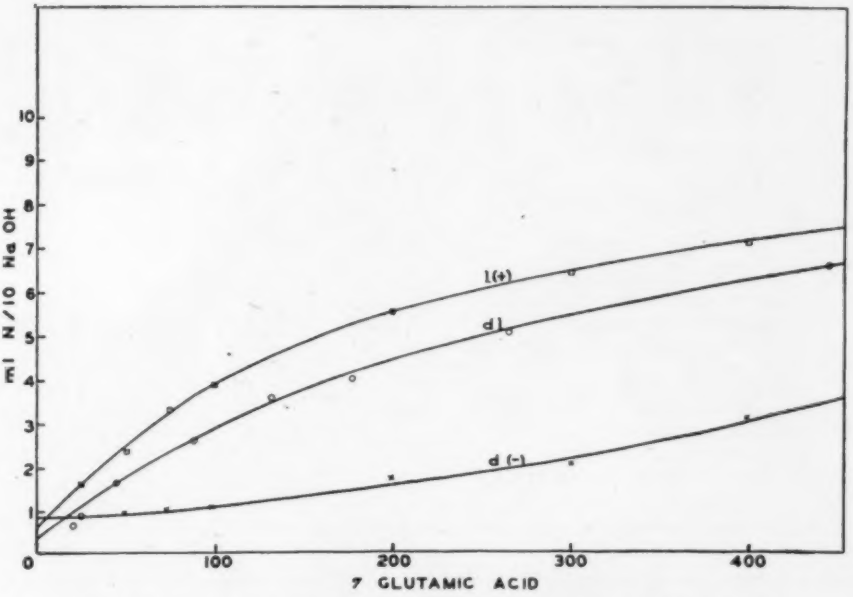

Fig. 5. Response of *L. acidophilus* to the different optical forms of glutamic acid.

Lactobacillus casei did not always give reproducible results and the standard curves were sometimes unsatisfactory. Therefore assays with this organism were considered less reliable than the ones obtained

TABLE I

GLUTAMIC ACID VALUES DETERMINED WITH VARIOUS MICROORGANISMS

Material analyzed	Nitrogen in dry feed material	Glutamic acid [1] in dry feed material as determined with:		
		L. arabinosus	*S. faecalis* R.	*L. casei*
	%	%	%	%
Grain and grain by-products				
Wheat	2.6	4.8	3.9	4.3
Granular wheat flour	2.4	4.7	3.8	4.1
Corn	1.5	2.6	2.1	1.9
Rye	2.9	4.2	3.6	4.0
Milo maize	1.9	3.2	2.6	2.7
Ground barley	2.9	3.8	3.2	—
Barley malt	2.4	3.6	2.5	2.5
Gluten feed	4.6	5.4	4.5	4.6
Gluten meal	7.1	10.2	9.4	7.7
Standard wheat middlings	3.7	5.0	4.5	4.4
Distillers' by-products				
Rye grains with solubles	5.1	6.9	5.5	7.0
Wheat grains with solubles	5.4	10.5	9.3	—
Milo grains with solubles	5.8	8.0	7.6	7.0
Corn grains with solubles	5.1	6.0	5.7	5.6
Corn solubles	4.5	4.7	4.3	4.6
Wheat-milo grains with solubles	5.2	9.7	7.7	8.7
Wheat-milo solubles	4.9	9.6	7.9	8.7
Vegetable protein concentrates				
Cottonseed meal	6.4	7.8	6.8	7.0
Soybean meal, extracted	9.1	10.7	9.0	9.8
Soybean meal, expeller	7.6	9.3	7.8	8.6
Animal by-products				
Tankage	11.3	9.2	7.9	9.5
Meat scraps	10.0	7.6	6.4	—
Liver meal	13.0	10.4	8.1	8.9
Liver powder	10.0	9.0	8.3	8.3
Miscellaneous feed supplements				
Animal feed yeast	8.3	6.6	5.4	5.9
Irradiated dried yeast	8.8	7.3	6.3	—
Dehydrated alfalfa	3.1	1.7	1.4	1.3
Dried whey	2.7	2.3	1.7	1.6
Dried cheese whey	2.5	2.1	1.9	—
Dried buttermilk	6.2	9.2	7.0	8.7
Dehydrated grass	3.1	3.8	3.8	—
Grass juice extract	5.3	1.4	1.5	—
Water-soluble fraction of grass	2.7	3.1	3.2	—

[1] The values were determined employing *l*(+)-glutamic acid as the standard.

with *Streptococcus faecalis* R. and *Lactobacillus arabinosus*. The values were included to indicate the trends of the results.

The assays with *Lactobacillus arabinosus* and *Streptococcus faecalis* R. are of high reproducibility; however, this does not imply that the results are accurate, as demonstrated by the response of the organisms

to the different optical forms of glutamic acid. The assays with *Streptococcus faecalis* R. determine the amount of *l*(+)-glutamic acid and are of high accuracy. The values obtained with *Lactobacillus arabinosus* must be considered as maximum values at the present time. It is to be hoped that assay methods for glutamic acid employing *Lactobacillus casei* can be improved so that the total amount of glutamic acid present in any material, irrespective of the optical configuration, can be more accurately determined.

Summary

The influence of asparagine and aspartic acid on the standard curve of glutamic acid was examined and the beneficial effect of asparagine in preventing sigmoidal curves was demonstrated.

The response of five organisms to the *l*(+), *d*(−) and *dl*-glutamic acid was investigated and its significance in glutamic acid analysis was discussed. A large number of feed materials was analyzed for glutamic acid and the results were reviewed in the light of the above findings.

Literature Cited

Baumgarten, W., Stone, L., and Boruff, C. S.
1945 Amino acid composition of grain alcohol fermentation by-products. Cereal Chem. **22**: 311–320.

Dunn, M. S., Camien, M. N., Rockland, L. B., Shankman, S., and Goldberg, S. C.
1944 Investigations of amino acids, peptides, and proteins. XVII. The determination of glutamic acid in protein hydrolysates by a microbiological method. J. Biol. Chem. **155**: 591–603.

Fischer, E.
1899 Ueber die Spaltung einiger racemischer Amidosäuren in die optisch-activen Componenten. Ber. **32**: 2451–2471.

Gunsalus, I. C., Niven, C. F., Jr., and Sherman, J. M.
1944 The identification of *Streptococcus lactis* R. as a strain of *Streptococcus faecalis*. J. Bact. **48**: 611.

Kibrick, A. C.
1944 The estimation of the dicarboxylic amino acids by titration. J. Biol. Chem. **152**: 411–418.

Kuiken, K. A., Norman, W. H., Lyman, C. M., Hale, F., and Blotter, L.
1943 The microbiological determination of amino acids. I. Valine, leucine and isoleucine. J. Biol. Chem. **151**: 615–626.

Lewis, J. C., and Olcott, H. S.
1945 A lactobacillus assay method for *l*(+)glutamic acid. J. Biol. Chem. **157**: 265–285.

Lyman, C. M., Kuiken, K. A., Blotter, L., and Hale, F.
1945 The microbiological determination of amino acids. II. Glutamic acid. J. Biol. Chem. **157**: 395–405.

Shankman, S.
1943 Amino acid nutrition of *Lactobacillus arabinosus*. J. Biol. Chem. **150**: 305–310.

Stokes, J. L., and Gunness, M.
1945 Microbiological methods for the determination of amino acids. I. Aspartic acid and serine. J. Biol. Chem. **157**: 651–659.

A PHOTOMETRIC EVALUATION OF THE "APPEARANCE" OF VARIOUS MALT SYRUPS AS INFLUENCED BY STORAGE CONDITIONS

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The observation has been repeatedly made that malt syrups undergo a gradual lightening in "color" followed by a progressive darkening as storage is prolonged. These changes in appearance are apparent by visual examination of the syrups. The darkening can be demonstrated with present methods for syrup color determination but the initial lightening cannot be detected.

Existing methods of measuring color in wort, beer, and malt syrups are dependent upon the amount of light transmitted through the sample. The color evaluation may be made visually, as with the Lovibond Tintometer, or electrically with a photocell. In any of these methods the stipulation is made that any turbidity in the solution must be removed prior to making the determination. As regards wort and beer, this may give valuable information, although Nissen and Peterson (1942) have shown that turbidity in beer must be considered if a true "appearance" value is to be obtained.

The determination of color in malt syrups presents further difficulties. According to the official method of the American Society of Brewing Chemists, a 10% solution is prepared, turbidity estimated in terms of haziness, and color determined with the Lovibond Tintometer on a filtered aliquot. When a malt syrup is diluted with water, the small protein particles flocculate, producing a very hazy solution. Consequently, any estimates made of the turbidity of the untreated syrup are seriously in error. The color determination on the filtered solution fails to compensate in any way for turbidity, and thus gives little information regarding the appearance of the original syrup.

The true appearance of a malt syrup is of primary importance when it is employed as the main ingredient of a product, as is the case in many pharmaceutical preparations. Obviously, a color value determined on a filtered solution of the syrup is of little value in standardizing the appearance of the finished product. However, when a malt syrup is used in relatively small percentages, as in dough supplementation, the true appearance of the syrup becomes a minor factor, and the usual color determination suffices to evaluate the syrup.

With the shortcomings of existing methods in mind, a new method was developed which yields values in agreement with the actual ap-

pearance of a malt syrup as determined by visual examination. The proposed method departs from existing methods for color determination in that reflected light is measured rather than transmitted light, and the determination is made on untreated malt syrup rather than on fractions of it obtained by dilution and filtration.

Method

The new procedure employes a Coleman Model 11 Universal Spectrophotometer set up as a fluorometer. The mercury lamp illuminator, with only the Corning 038 filter in place, is mounted on the photometer so that the light falls directly on top of the cell containing the syrup. This cell [1] has an inside diameter of 20 mm and is 30 mm in height. The Coleman PC-5 filter is inserted between the sample and the photocell. With such an optical system, the light striking the photocell is chiefly that reflected from particles within the syrup. The amount of reflected light increases as the number of particles (turbidity) increases, and decreases as the color deepens, owing to a reduction in transmission of light within the sample.

For the determination of the appearance of a malt syrup, the cell is filled with the sample, care being taken to avoid the inclusion of air bubbles, and the excess syrup struck off with the edge of a spatula. The cell is placed in position under the illuminator and, with the drum dial set at zero optical density, the galvanometer is adjusted to zero. The syrup is then removed and a cell containing exactly 8 ml of distilled water is placed in position. The drum dial is rotated until the galvanometer again reads zero. The optical density is read from the drum dial, multiplied by 100, and that value subtracted from an arbitrary figure of 65.0. The resulting values have been termed "appearance units."

If the syrup is so dark that the galvanometer cannot be set to zero when the drum dial is at zero optical density, an alternate procedure may be used. With the galvanometer control at the extreme clockwise position and the drum dial set at zero transmission, the sample is inserted and the reading taken from the galvanometer. A similar reading is then made with the water standard. If the syrup reading is lower than the water, the difference between the two is added to 75.0. If the syrup reading is higher, the difference is subtracted from 75.0. The constant, 75.0, was calculated from data obtained on malt syrups whose appearance values could be determined with both procedures. (The ranges of the two methods overlap slightly at the transition point.) This procedure, while not as sensitive as the method for lighter syrups,

[1] May be obtained from Wilkens-Anderson Company, Chicago, Illinois.

has been used in following the aging of a malt syrup. The transition between the two procedures is quite smooth.

Experimental

A comparison was first made of the color values obtained by the official method and the appearance units obtained with the proposed method. For this comparison a sample of a diastatic, all-barley malt syrup was stored at 100°F (37.8°C) and readings were made at intervals with the new method and with the Lovibond Tintometer. Figure 1 presents the data obtained in this study.

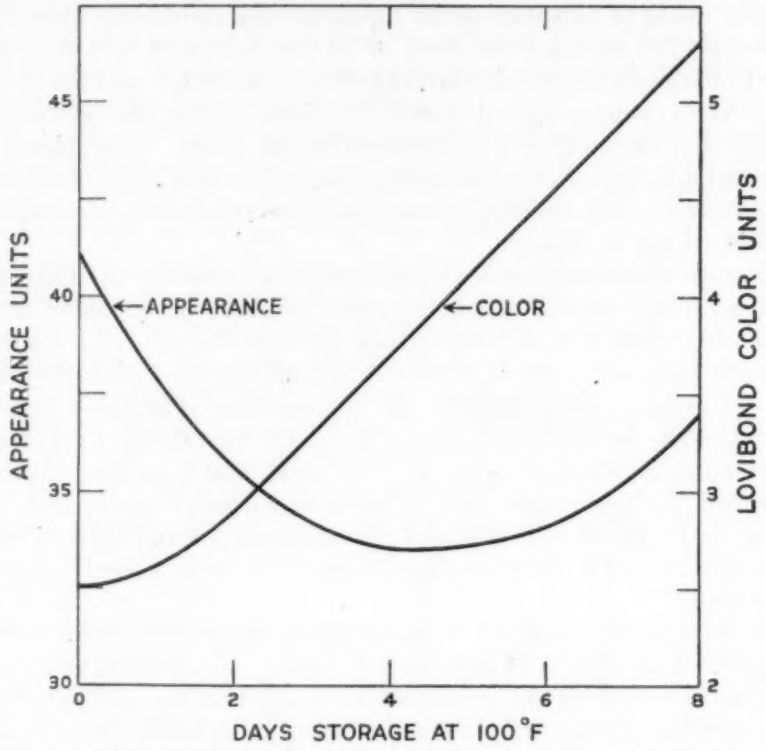

Fig. 1. Comparison of appearance and color readings on a malt syrup stored at 100°F (37.8°C).

Figure 1 shows that the new photometric method follows accurately the initial lightening in appearance and the subsequent darkening as observed visually. The Lovibond readings (determined on a filtered aliquot of a 10% syrup solution) show only a continuous increase in color from the beginning of the test.

It has been found that the new method will readily differentiate between two syrups that are only just perceptibly different by visual examination.

With a method available which closely measures the appearance changes in a malt syrup, further experimental work was undertaken to apply it to various types of syrups kept under different storage conditions and to determine the reasons for the observed changes.

To study the effect of the type of syrup upon the appearance changes during storage, samples of five different types (determined by variations in mashing procedure and mash bill) of syrups were stored at

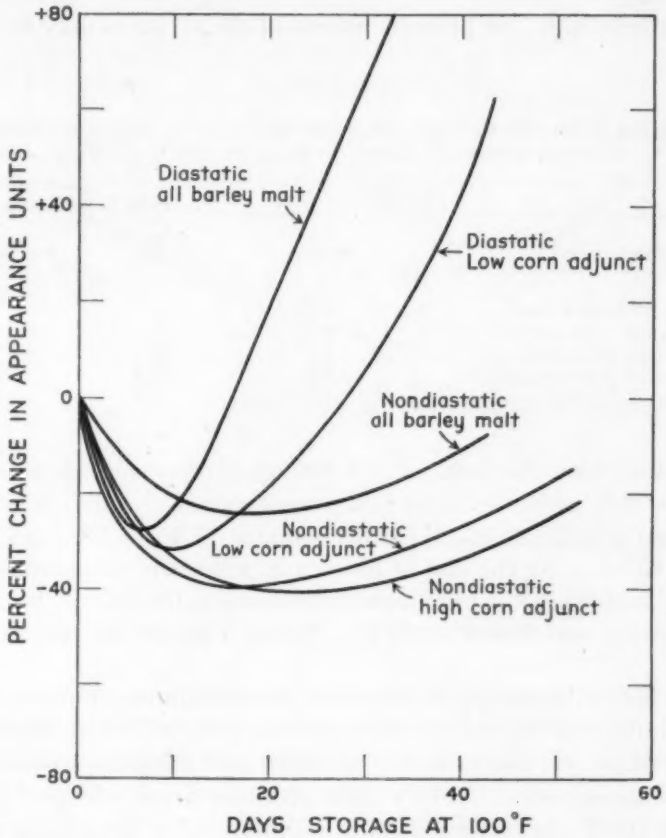

Fig. 2. The effect of syrup composition upon the appearance changes during storage at 100°F (37.8°C).

100°F (37.8°C) and the appearance changes measured at intervals. Complete analyses were made of the syrups before and after the storage period. The only significant changes shown were reductions in pH, increases in acidity, and reductions in the Lintner values of the diastatic syrups, confirming data reported by Graber (1914). The data on the appearance changes are presented in Figure 2.

All syrups show the initial lightening in appearance, confirming visual observation. The most striking feature shown by these curves

is the effect of the nature of the syrup upon the darkening process. The diastatic syrups reach a minimum appearance value much sooner than the nondiastatic types and then rapidly increase in color. An increase in the ratio of adjunct to malt causes a corresponding increase in the storage time required to produce a minimum appearance value, indicating that darkening is less rapid. Figure 2 shows that a low-corn malt syrup begins to darken in about 16 days as compared with 30 days for the high-corn malt syrup. The rate at which darkening takes place is closely related to the protein content of the syrup as may be seen in Table I.

TABLE I

RELATIONSHIP BETWEEN PROTEIN CONTENT OF MALT SYRUPS AND APPEARANCE CHANGE AFTER 30 DAYS' STORAGE AT 100°F (37.8°C)

Type of malt syrup	Protein	Change in appearance units	
		Original value	Value after 30 days
	%		
Diastatic, all-barley malt	7.3	42.5	70.5
Diastatic, low-corn adjunct	4.7	30.5	32.3
Nondiastatic, all-barley malt	4.3	66.5	53.2
Nondiastatic, low-corn adjunct	3.9	52.5	34.5
Nondiastatic, high-corn adjunct	2.2	45.0	27.0

To determine the influence of storage temperature, four samples of a diastatic, all-barley malt syrup were stored at different temperatures; two samples at 100°F (37.8°C), one at 75°F (23.9°C), and one at 35°F (1.67°C). At the end of four days, when the two syrups stored at 100°F had reached a minimum appearance value, one of the samples was removed and stored at 35°F. Figure 3 shows the results of this test.

The initial lightening is apparent in all samples, but the rate is greatly influenced by storage temperature. At 35°F both the lightening and darkening processes are inhibited and the syrup remains very stable in appearance. At 75°F both processes are accelerated slightly, while at 100°F they proceed quite rapidly. It is interesting to note that the sample which was allowed to reach a minimum appearance value at 100°F and then removed to the 35°F chamber showed no darkening, but remained at the same low level.

It is quite apparent from the data presented, which confirm visual observations, that the change of appearance of a malt syrup progresses in two stages: an initial lightening followed by an increase in color.

It is generally known that substances containing significant amounts of reducing sugars and the lower degradation products of protein hydrolysis are subject to darkening during storage. This increase in

color is attributed to the formation of pigments called melanoidins. The nature and behavior of this reaction have been reported by Maillard (1912), Enders (1938), Enders and Theis (1938), and Brown and Nissen (1942). All have shown that the rate of color development is dependent upon the amounts of reacting substances present and on temperature.

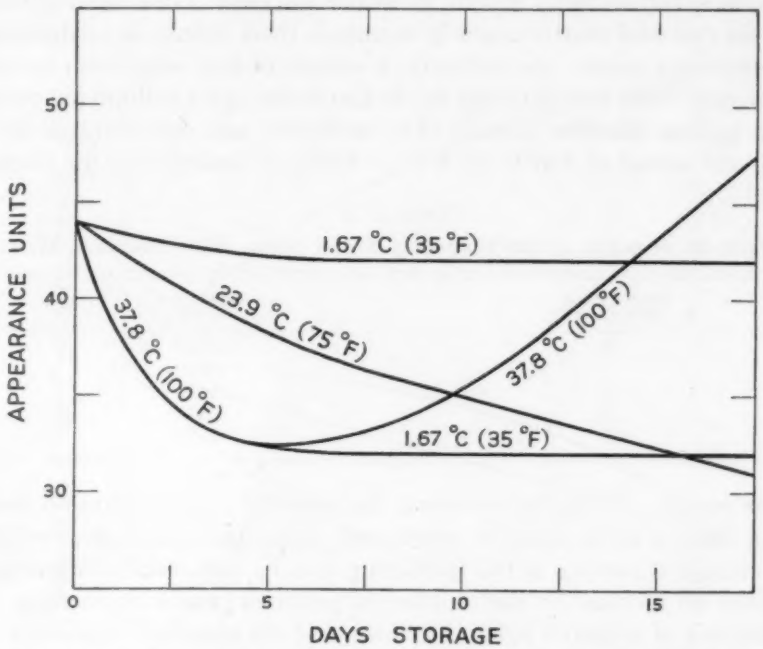

Fig. 3. The effect of storage temperature upon the appearance changes of a diastatic, all-barley malt syrup.

To determine whether the lightening phenomenon occurs at higher temperatures, such as previously employed in connection with the study of melanoidin formation, a sample of diastatic, all-barley malt syrup was placed in a drying oven at 212°F (100°C). Appearance measurements were made at approximately 20-minute intervals. The syrup showed the same lightening and darkening phenomena but at a greatly accelerated rate, reaching a minimum value in 1.25 hours and an extremely dark and foaming condition in 2.5 hours. Thus, even at high temperatures, the lightening process occurs although it is quickly masked by the rapid melanoidin formation.

Since it is generally accepted that the melanoidin condensation is responsible for the darkening of malt syrups, no further investigation was thought to be needed on that phase of the appearance changes. The initial lightening, however, appears to be a change not previously

reported, and so further study was made as to its nature and influencing factors.

It has been established by the data shown in Figure 1 that dilution and filtration of a malt syrup remove the constituents responsible for the decrease in appearance units. These constituents are quite evidently those which produce turbidity in a syrup. Since the turbidity in a malt syrup is chiefly protein in nature and exists in a colloidal state, if all the colloidal matter could be removed, there should be no decrease in appearance units. Accordingly, a sample of first wort from an all-barley malt brew was dialyzed for 24 hours through a cellophane membrane against distilled water. The dialysate was concentrated to a syrup and stored at 100°F (37.8°C). Table II summarizes the results

TABLE II

EFFECT OF STORAGE AT 100°F UPON A SYRUP MADE FROM DIALYZED WORT

Days storage at 100°F (37.8°C)	Appearance units
0	61.0
1	61.0
2	61.0
5	65.0
7	74.0

of this study. Thus, by removing the colloidal material from a malt syrup, there is no decrease in appearance units during storage, and the only change occurring is the darkening due to melanoidin formation.

Since an increase in the number of particles causes an increase in the amount of reflected light, it follows that the observed lightening of a malt syrup is undoubtedly due to increased turbidity, caused by an aggregation process in which protein molecules collect together to form micells large enough to reflect light.

Kruyt (1926) has shown that the rate of aggregation varies directly with temperature and inversely with the viscosity of the dispersion medium. The storage data, previously presented, show very clearly the effect of temperature upon the rate of the decrease in appearance units. Since the viscosity of a syrup depends greatly on the temperature, the observed differences in the rate of lightening at various storage temperatures undoubtedly are influenced by the accompanying viscosity variations.

To determine the effect which the viscosity of the syrup has on the rate of appearance changes, various additions of Irish moss were made to aliquots of a thin, all-barley malt syrup and each portion concentrated to about 42°Be. The Irish moss was added in amounts equivalent to 0.5%, 1.0%, and 2.0% of the solids content of the thin syrup.

Figure 4 shows the effect of increasing the viscosity on the rate of change of the appearance values. The data show that if the viscosity is increased to a sufficient degree, there is no aggregation and consequently the syrup does not show the characteristic lightening in appearance.

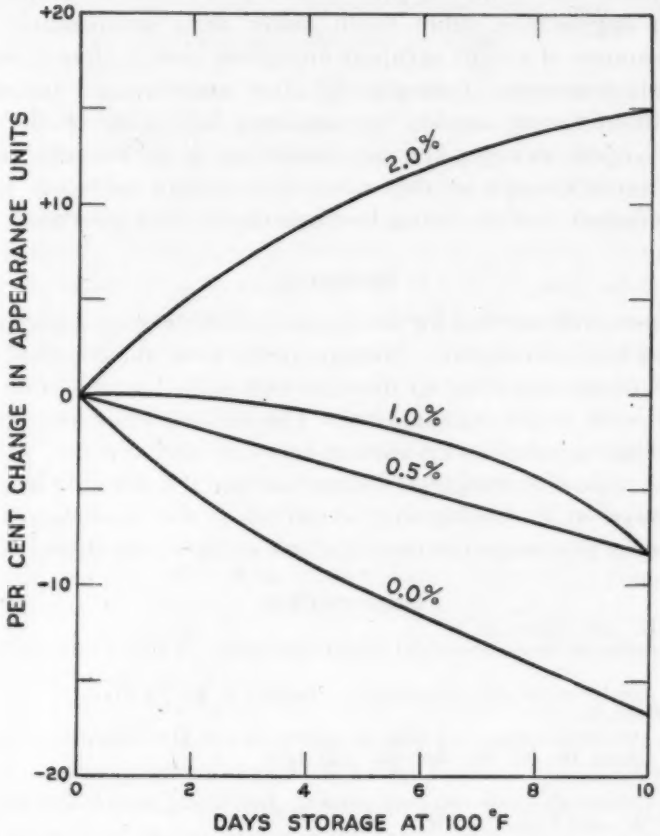

Fig. 4. The effect of adding Irish moss upon the appearance changes of a malt syrup stored at 100°F (37.8°C).

Discussion

The appearance changes undergone by a malt syrup are caused by two concurrent phenomena. The lightening effect is quite evidently a physical process during which the protein system, by aggregation, changes from a molecular to a micellar state. The subsequent darkening, on the other hand, is considered to be a chemical reaction between reducing sugars and protein hydrolysis products. The rate of decrease in appearance units depends upon storage temperature and viscosity. The darkening effect is related to temperature, amount of protein

present, the extent to which that protein is degraded, and to the nature of the reducing sugars. For example, Haman and Fosnot (1944) have found that diastatic syrups having a high protein and amino acid content, as well as a considerable amount of dextrose, show the most rapid melanoidin formation. This rapid color formation quickly masks the lightening process and thus diastatic syrups reach a minimum appearance value much faster than nondiastatic types. The appearance of a malt syrup at any given time is thus a resultant of the two processes. Immediately after manufacture, turbidity is the dominant factor causing an apparent lightening of the syrup. However, upon storage, the accumulation of melanoidin pigment becomes great enough so that color rather than turbidity has the greater influence and the syrup becomes darker in appearance.

Summary

A photometric method for the evaluation of the appearance of malt syrups has been developed. Measurements were made on the syrups "as is" without resorting to dilution and filtration, and the results correlated with visual examination. The method has been applied to a series of storage studies on various types of malt syrups.

All the syrups investigated showed an initial lightening in appearance followed by darkening with variations in rate and degree owing to differences in storage temperature and syrup composition.

Literature Cited

Brown, E. B., and Nissen, B. H.
1942 Effect of temperature and age on malt syrup. Cereal Chem. 19: 243–248.
Enders, C.
1938 Zur Kenntnis der Melanoidine. Kolloid Z. 85: 74–87.
—— and Theis, K.
1938 Die Melanoidine und ihre Beziehung zu den Huminsäuren. Brennstoff Chem. 19: 360–365, 402–407, 439–449.
Graber, H. T.
1914 Laboratory studies on malt extract. Ind. Eng. Chem. 6: 403–404.
Haman, R. W., and Fosnot, R. H.
1944 Unpublished data.
Kruyt, H. R.
1926 The mechanism of coagulation. In: Alexander, Jerome, Colloid Chem. 1: 306–322. Chemical Catalog Co., Inc., New York.
Maillard, L. C.
1912 Chimie organique. Action des acides amines sur les sucres; formation des melanoidines par voie methodique. Compt. rend. 154: 66–68.
Nissen, B. H., and Peterson, R. B.
1942 Color and turbidity in beer and wort. American Society of Brewing Chemists Proceedings, 77–90.

COMPARISONS BETWEEN SOME PROPERTIES OF MIXOGRAMS FROM FLOUR AND UNSIFTED WHOLE WHEAT MEAL

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Many cereal chemists are familiar with mixogram studies, which have appeared in profusion of late years in the pages of *Cereal Chemistry*, and there is little need to make more than passing reference to these in the present paper. Johnson and Swanson (1942) and Lamb (1944) have reported mixogram studies made with sifted wheat meal with the object of discovering if this material could be used for determining the varietal pattern and eliminate the necessity of milling the wheat to flour. Their conclusions indicated that sifted wheat meal could, with some restrictions, be employed in lieu of flour in securing mixogram patterns, but it seemed to the present writers that the time and labor required to obtain a suitable sifted wheat meal was little less than that needed to produce experimentally micro-milled flour. Micro-milled flours have been shown to compare quite favorably with flours produced in the Allis-Chalmers experimental mill, and can be employed to assess wheat quality with resultant economy in material and time (Geddes and Aitken, 1935; Sibbitt, Scott, and Harris, 1943). It is probable that slightly less wheat would be required for the sifted meal method, however, and this might be a factor of some importance in wheat breeding work.

In view of the favorable reports on the value of mixograms made from sifted whole wheat meal, the authors decided to investigate the possibility of using unsifted whole wheat meal as produced in a small experimental grinder for nitrogen content determinations. It appeared that a distinct saving in time and labor would be effected by using this procedure rather than working with micro-milled flours, which require 30 minutes to mill and at least 75 g of wheat. Pertinent curve properties secured from unsifted wheat meal and flour could be compared, and the utility of wheat meal mixograms for detecting varietal and station differences could be determined.

Experimental

Materials. The principal series of wheats employed in this study consisted of nine hard red spring varieties grown in experimental plots at five North Dakota stations in 1944. This series, denoted as the "plot series," include those of major agricultural importance in the

state. In addition, several other varieties grown in experimental plots at Fargo were included as well as a number of small samples from the wheat nursery. This set of samples is denoted as the "entire series." The wheats were all sound and free from any damage which might have tended to vitiate the results. The data from the plot series were used to compare the influence of variety and station on flour and wheat meal mixogram properties, while the data from the entire series were employed to determine the relation between mixogram characteristics secured from flour and wheat meal on diverse wheats grown under the same climatic environment. Wheat protein varied from 10.7 to 16.0% with an average of 13.7% for all samples, while for the plot series the corresponding values were 10.7, 15.0, and 13.4% respectively. Another set of samples, consisting of 35 durum wheats and the resultant semolinas, was also examined.

Methods. A small electrically driven coffee mill with conical grinding plates was used for grinding the whole wheat meal. This appliance cleans quite satisfactorily between samples without dismantling, and is rapid and efficient. The material was put through the grinder three times to attain satisfactory fineness, after which it was thoroughly mixed since the branny portion tends to separate. This procedure is followed in this laboratory in the preparation of samples for nitrogen assay. The granulation of the material was quite uniform as found by bolting a number of samples through various-sized flour sieves and weighing the several separations. Before grinding in the coffee mill, the wheat was passed once through the rolls of a Tag-Heppenstall moisture tester to break the kernels and facilitate grinding. Semolina prepared by the usual method (Harris and Sibbitt, 1942) was reduced to the granulation of bread wheat flour in the micro flour mill, since this material has been found to be more suitable for mixogram investigations than unground semolina. The wheat meal was prepared as in the instance of the hard red spring wheat samples. To ascertain the effect of meal particle size on the curve pattern, a composite sample of the wheat, ground as described, was bolted using three sieves of the following sizes: 8 XX, 50 GG, and 40 GG. It was found that 28.2% of the total passed through the 8 XX and 22.4% was retained, while 22.4% was retained on the 50 GG and 27.0% on the 40 GG.

The wheats from the various plots were milled on an Allis-Chalmers experimental mill, while the nursery samples were milled on a micro mill. The flow sheets and techniques involved have been adequately described by Sibbitt, Scott, and Harris (1943). Long patent flours were produced in both mills. A constant absorption of 56% was used in securing the hard red spring wheat meal mixograms. This value

was obtained after a number of trials involving different absorption levels, and appeared to be quite satisfactory in revealing curve pattern differences. Apparently, wheat meal is not as responsive to absorption variations as flour. For the flour, the absorption used was that determined when baking.

The procedures employed in obtaining mixograms and their dimensions were described by Harris, Sibbitt, and Elledge (1944) and Harris, Sibbitt, and Scott (1945). It was pointed out in these papers that hard red spring wheat mixograms are most informative when secured at a temperature of 30°C, and this temperature was used for both the flour and wheat meal studies. No difficulty was experienced in securing normal curves from the wheat meal. A constant absorption of 60% was used for the durum wheat meal mixograms, while a varied absorption calculated from the macaroni processing data was used for the semolina. After a substantial amount of preliminary work at various temperatures, 15°C was chosen as giving a satisfactory curve, and was used throughout in obtaining the durum wheat and semolina mixograms. Distilled water was used in all the mixes.

Results and Discussion

The data from the entire series will be discussed first, with comparative statistics obtained from the wheat and flour mixograms. This will be followed by a comparison of variety and station influences on the curve characteristics of the wheats and flours.

Statistical Comparisons from the Wheat and Flour Data. Table I shows minimum, maximum, and mean values for the three mixogram

TABLE I

COMPARATIVE WHOLE WHEAT MEAL AND FLOUR DATA SECURED FROM THE TWO SERIES OF SAMPLES

Mixogram property	Material used	N[1]	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
			cm	*cm*	*cm*	*cm*	%
Dough development stage	Wheat	45	3.2	7.5	5.2	1.32	25.2
		77	2.1	11.9	4.9	1.43	29.1
	Flour	45	3.2	8.6	5.7	1.30	22.9
		77	1.9	8.6	5.0	1.60	32.1
Range of dough stability	Wheat	45	2.3	9.5	4.9	1.30	26.4
		77	2.3	16.4	5.0	1.81	36.0
	Flour	45	2.8	10.9	5.8	2.01	34.4
		77	1.7	10.9	5.2	2.07	39.5
Curve height	Wheat	45	6.1	8.5	7.4	0.61	8.2
		77	5.2	8.6	7.5	0.60	8.0
	Flour	45	5.4	8.2	6.9	0.71	10.4
		77	5.4	8.7	7.2	0.79	11.0

[1] N (plot series) = 45; N (entire series) = 77.

properties examined, with standard deviations and coefficients of variation. The correlation coefficients between the wheat and flour properties are given in Table II. These tables contain the plot series data as well as the entire series which includes the plot series and additional plot and nursery samples. It is apparent that the inclusion

TABLE II

CORRELATION COEFFICIENTS FOR THE MIXOGRAM DATA

		Variables correlated		
Mixogram property	N	X	Y	r_{xy}[1]
Dough development stage	45	Wheat	Flour	+0.524
	77	Wheat	Flour	+0.649
Range of dough stability	45	Wheat	Flour	+0.633
	77	Wheat	Flour	+0.414
Curve height	45	Wheat	Flour	+0.681
	77	Wheat	Flour	+0.692
	77	Wheat	Wheat protein	+0.714
	77	Flour	Wheat protein	+0.769

[1] N (plot series) = 45; value of r_{xy} at 5% point 0.304; at 1% point 0.398.
N (entire series) = 77; value of r_{xy} at 5% point 0.224; at 1% point 0.292.

of the extra samples grown at Fargo somewhat increased the variability of the wheat meal dough development stage and range of dough stability measurements. There were no marked differences between the mean wheat and flour values, nor consistent differences in variability, though there was a tendency for the flour to yield better differentiation in mixogram properties. All the correlation coefficients are significant at the 1% point, but are not sufficiently large to permit very accurate prediction of flour mixogram patterns from a knowledge of wheat meal mixogram properties, although the correlations do show a relatively close association between the properties of wheat and flour mixograms.

Comparisons between Varieties and Stations. The mean values for the mixogram properties for wheat varieties and stations are shown in Table III for both wheat and flour. Figure 1 presents representative curves secured from the whole wheat meal and the flour mixes. A general similarity in curve pattern is evident between the wheat and flour curves. The long dough development stage and range of dough stability of Vesta are apparent in both wheat and flour mixograms, as contrasted with relatively short corresponding values for Mida and No. 3103. Thatcher and Cadet produced curves in both the wheat and the flour that were similar in appearance.

TABLE III

VARIETAL AND STATION AVERAGES FOR WHEAT PROTEIN CONTENT AND MIXOGRAM PROPERTIES

(Arranged in order of increasing wheat protein content)

Varieties or stations	Wheat protein	Dough development stage		Range of dough stability		Curve height	
		Wheat	Flour	Wheat	Flour	Wheat	Flour
	%	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
	VARIETY AVERAGES						
Vesta	12.9	6.0	7.1	6.3	6.7	7.4	7.0
Pilot	13.1	4.9	5.7	4.8	5.3	7.3	6.7
Thatcher	13.2	5.5	5.6	5.0	6.3	7.5	6.8
Mida	13.3	4.0	5.2	4.5	5.2	7.1	6.8
Rival	13.6	4.8	5.1	4.5	5.6	7.3	7.3
Cadet	13.6	6.2	5.7	5.5	6.4	7.9	7.0
3103	13.6	4.3	4.5	3.6	3.8	7.6	7.3
Regent	13.7	4.9	5.5	4.6	5.6	7.8	7.1
Newthatch	14.0	4.8	5.9	4.9	6.5	7.6	6.8
[illegible]	STATION AVERAGES						
[illegible]							
Fargo	11.8	6.1	6.2	5.8	8.6	6.8	6.0
Dickinson	12.9	4.6	5.1	3.8	5.2	7.4	7.0
Edgeley	13.8	5.1	5.7	4.9	5.3	7.5	7.0
Williston	14.2	4.3	4.1	5.0	3.8	8.1	7.7
Langdon	14.4	5.2	6.9	4.7	5.7	7.7	7.1

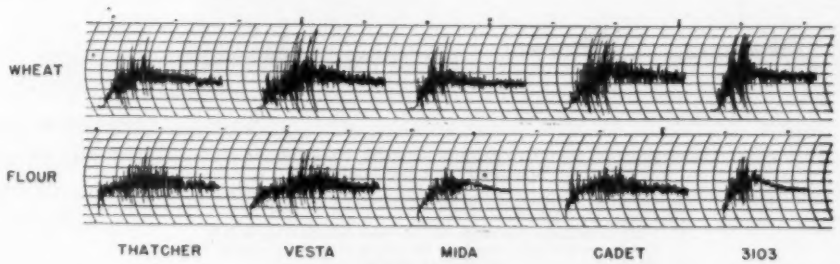

Fig. 1. Representative mixograms of five hard red spring wheat varieties made from the whole wheat meal and flour.

Table IV gives analyses of variance of the wheat and flour mixogram data from the plot series. Very significant differences are shown for all properties, except curve height between varieties for the flour. Little difference is to be noted between the wheat and flour variances for stations. The flour variances are much larger than those of the wheat meal between stations, indicating that flour is possibly more

sensitive to differences in mixogram properties caused by environment during wheat growth than is wheat meal. Otherwise there is no indication in these data that flour is more suitable than wheat meal for

TABLE IV

ANALYSES OF VARIANCE OF THE MIXOGRAM DATA FOR 45 PLOT SAMPLES GROWN IN 1944

Source of variation	Degrees of freedom	Dough development stage		Range of dough stability		Curve height	
		Wheat	Flour	Wheat	Flour	Wheat	Flour
Between varieties	8	2.64**	2.37**	2.84**	3.97**	0.34**	0.27
Between stations	4	4.04**	10.64**	4.64**	27.71**	1.84**	3.65**
Interaction (error)	32	0.56	0.48	0.66	1.05	0.08	0.13
Total	44						

** Denotes significance exceeding 1% point.

determining the mixogram pattern. Some of the variability in dough stability may be due to variations in protein content, especially the low protein content of the Fargo samples. In this instance, the height of the curves was decreased by the low protein wheats with a resultant increase of the dough stability measurement.

Comparisons among Wheat Meal Fractions. The mixogram patterns obtained from the four separations into which the whole wheat meal was divided are shown in Figure 2. Distinct changes in curve

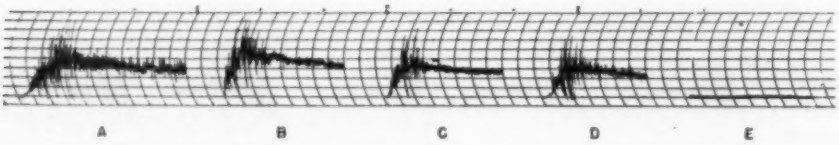

Fig. 2. Mixograms obtained from the ground whole wheat meal and the four fractions separated from this sample.

A. Ground; unsifted whole wheat meal.
B. Flour; throughs from 8 XX.
C. Fine middlings; through 50 GG and retained on 8 XX.
D. Coarse middlings; through 40 GG and retained on 50 GG.
E. Bran and shorts; retained on 40 GG.

pattern are apparent among the different separations. The material retained on the 40 GG sieve which was principally bran and shorts would not form a dough. Therefore, the curve produced was caused merely by the drag of the pins in the mixer head through the material in the bowl. The other curves were typical and very similar to what was expected from material of this type. It is interesting to note that the two curves made from the "middlings" material, which contained some bran and shorts, had the peculiar level stretch or dip, at the start

of the mixogram, which is common and consistent in wheat meal curves.

Comparisons with Durum Wheats. Comparisons were also made between the ground durum wheats and reduced semolina. The usual curve measurements were made but the data, on critical examination, showed no relations of value. Erratic and unreliable results were secured from the wheat meal curves, and no useful purpose would be served by presenting them. Distinct varietal differences were evident, however, in the semolina curves, but owing to lack of variety replication at different stations a statistical analysis of these differences could not be made.

Summary

Comparisons between mixograms made from ground unsifted whole wheat meal and experimentally milled long patent flour showed very significant relations for all mixogram dimensions examined. However, the relationships were not sufficiently high to permit the prediction with accuracy of the properties of flour curves from those of the meal curves. There were no marked differences in means or variability between the wheat and flour values.

An analysis of the data representing nine wheat varieties grown at five stations showed little advantage in employing flour rather than wheat meal in differentiating between varieties in respect to mixing properties. However, flour mixograms showed greater variations between stations, although very significant differences were found between stations for the meal data.

The results indicated that mixogram patterns obtained from unsifted ground whole wheat meal as employed for nitrogen determinations could be quite successfully employed for assessing varietal mixing requirements, particularly in sorting out wheats differing rather markedly in these properties. Thus a substantial saving in time and amount of wheat necessary for a test could be effected by the proposed procedure.

Acknowledgments

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Literature Cited

Geddes, W. F., and Aitken, T. R.
1935 An experimental milling and baking technique requiring 100 grams wheat. Cereal Chem. 12: 696–707.

Harris, R. H., and Sibbitt, L. D.
1942 Experimental durum milling and processing equipment, with further quality studies on North Dakota durum wheats. Cereal Chem. 19: 388–402.

——, ——, and Elledge, M.
1944 Varietal, station, and seasonal effects upon some properties of mixograms made from hard red spring wheat flours by various mixing methods. Cereal Chem. **21**: 38–48.
——, ——, and Scott, G. M.
1945 Comparative effects of variety and environment on some properties of North Dakota hard red spring wheat flours. Cereal Chem. **22**: 75–81.
Johnson, J. A., Jr., and Swanson, C. O.
1942 The testing of wheat quality by recording dough mixer curves obtained from sifted wheat meals. Cereal Chem. **19**: 216–229.
Lamb, C. A.
1944 Sifted wheat meal mixograms for selecting soft wheat varieties. Cereal Chem. **21**: 57–64.
Sibbitt, L. D., Scott, G. M., and Harris, R. H.
1943 A further comparison of flours obtained with the micro and Allis-Chalmers mills. Cereal Chem. **20**: 679–685.

ON A FACTOR IN OAT BRAN MARKEDLY ANTAGONISTIC TO BAKING QUALITY OF WHEAT FLOUR

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During the war, oats have been used in grists in the United Kingdom to replace a certain proportion of wheat. For reasons already given (Jones, Simpson, and Kent, 1943), the use of "green" or raw groats, i.e., groats from unkilned oats, was preferred to that of whole oats. The groat is like the wheat grain in that it has been freed from the chaff, hull, or husk (which firmly encloses the groat in the ordinary grain of oat) but is enclosed within its own bran coat. This appears less robust than that of the wheat grain—as might be expected from the protection afforded by the more firmly attached chaff—but is probably not significantly less in amount, relative to the endosperm. It is abundantly covered with fine hairs which, desirably, are removed by scouring before milling.

The analytical figures shown in Table I have been determined on green groats and their products. The groat products were made on a laboratory mill using apparatus and methods substantially the same as those employed for wheat flour milling. In milling groats most of the endosperm is obtained as break flour, i.e., flour liberated during the opening of the grain by the fluted rolls. The fiber content of the flours was determined by the method officially used for National flour in Great Britain as recommended by the Analytical Methods Committee (1943). Oil was determined by extraction under a reflux condenser for 24 hours with boiling petroleum ether (B.P. 40°–60°C).

The range of published values for composition of oats, groats, and various commercial oat products has been summarized by Moir (1942), who does not, however, give data for the oat husk, as such. Kent-Jones (1939), citing Berry, gives: moisture, 6.8; protein, 2.45; oil, 1.27;

TABLE I

ANALYTICAL RESULTS ON GREEN GROATS AND THEIR PRODUCTS

(14.0% moisture basis)

Sample	Fiber	Oil	Ash	Total phosphorus	Phytate phosphorus
	%	%	%	mg/g	mg/g
Whole groats (from Scotch oats)	1.4	6.9	1.87	3.99	2.64
Whole groats (from Welsh oats)	—	—	—	4.29	2.76
Groat bran (sample 1), approximately 15% by weight of groats milled	3.7	8.6	—	—	5.85
Groat bran (sample 2), approximately 15% by weight of groats milled	3.55	9.2	—	—	—
Straight-run groat flour:					
Sample 1, of 55% extraction	0.28	5.0	0.84	1.93	0.79
Sample 2, of 65% extraction	0.25	—	—	—	—
Sample 3, of 66% extraction	0.22	5.1	—	—	—
Sample 4, of 70% extraction	—	5.1	—	—	—
Sample 5, of 85% extraction	—	5.6	—	—	—
Groat flour streams, together constituting the 66% extraction (straight-run sample No. 3 above):					
Break flour =39% of groats	0.15	4.65	—	—	—
High grade reduction flour =15.5% of groats	0.25	5.4	—	—	—
Low grade reduction flour =11.5% of groats	0.43	6.45	—	—	—

ash, 3.9; and fiber, 33.5% in the husk. On a 14% moisture basis the writers found 30.2% fiber and 0.3% oil in the oat husk. The oil value is thus much lower than that reported by Berry, but his figures for the oil content of oats, husk, and groats do not give a satisfactory balance. Although the husk is very fibrous, the fiber content of groats and groat bran is lower than with wheat. However, commercial samples of green groats vary appreciably in fiber content according to the proportion of residual husk they contain and the degree of scouring effected in dehulling.

The wartime use of green groats as part of a breadmaking grist is seriously complicated by the effect on baking quality. The effect was explored extensively, using flours and meals prepared from groats in the laboratory and also flour commercially milled from mixtures of groats and wheat. The baking test was a straight dough system

using 800 g (1¾ lb) samples of flour with 2% yeast, 1.25% salt, and a total fermentation time, including final proof, of 4 hours at 80°F. The doughs were mixed by hand as is common in British laboratory practice, no process of mechanical development being applied. They were divided at 2¼ lb to give a finished loaf of 2 lb. The typical effects to be described were equally well observed when the baking system was varied by changing the proportion of water used in dough-making, by varying the fermentation time, or by the use of improvers.

In the first place, the effect was studied of adding groat wholemeal, ground to flour fineness, in various percentages to high-quality, London-milled 85% National flour (June 1942). The properties of the dough were found to be only slightly affected. There was a slight increase in the stickiness of the dough whose top tended to become flat as fermentation proceeded, but apart from this slight stickiness the dough handled normally when moulded after fermentation. On the other hand, the additions had a markedly depressing effect on loaf quality, even in small amounts, producing a pronounced fall in volume, together with an opening and coarsening of the grain. The trend in loaf volume is shown in the following:

% of groat meal in blend	Loaf volume (cc per 2 lb loaf)	Fall in volume as % of volume of control loaf
0 (control)	3,138	—
1	2,920	7.0
2	2,644	15.7
3	2,554	18.6
4	2,447	22.0
5	2,408	23.3

In general, the effect on crumb characters ran parallel with the fall in volume. There was very distinct deterioration even at 1%. At 2% the greater part of the total drop in crumb quality had taken place.

The affect appears to be quite general, having been found to similar extents with various groats from oats grown in different parts of the United Kingdom, blended with various 85% National flours. With a 5% blend the fall in loaf volume in every case was about 20% or a little over. In one or two cases 10% blends were tried, the volume drop being about 30%.

Later tests have shown parallel effects when the additions were made to wheat flours of 80% extraction.

The effect of oat flours of various extractions, milled as described above from green groats, was found to depend characteristically upon the extraction of the groat flour. A flour of short extraction, 70% or less, when blended up to 5% or so with wheat flour, did not appreciably affect its baking quality. At 10%, however, there was a 5 to 7% fall in volume and a definite deterioration in crumb quality. As the

extraction of the oat flour was extended from 70 to 100%, its effects on baking quality when used at the 5% level became progressively more apparent. The data supporting this summarized statement were given and discussed in the cited publication by Jones *et al.* (1943). Groat flours of extraction not higher than 60 to 70% may be made reasonably free from groat bran, but owing to the difficult milling properties of this cereal, the selection between endosperm and bran becomes rapidly less sharp as the extraction is raised to 85% and above. This suggested that the groat bran must be responsible for the deteriorative effect, and the present authors found that the addition to wheat flour of groat bran only, in proportions equivalent to its presence in a blend containing 5% of ground groats, substantially reproduced the deteriorative effect of the latter.

The experiment was made several times, using bran remaining after the separation of about 70% of flour and 15% of fine offals from different lots of groats. The effect was found to be no less reproducible than that of the ground groats. In a 5% addition of the latter the proportion of material corresponding to a separated groat bran would be about 1 to 1.5% of the entire blend. Tests in which 1, 1.5, and 2% of ground groat bran alone were added to the wheat flour showed the effect to be progressive. It was marked at 1%; at 2% it resembled, and was fully as great as, the drop caused by 5% ground groats. At 1.5% the deterioration in crumb quality was in line with the latter, but the fall in volume slightly less.

The cleaner the groat bran, i.e., the more thoroughly it had been freed from adhering endosperm, the greater was the effect. It should also be mentioned that when groats were bisected transversely the halves containing the germ gave the same effect as those containing the beard end. The effect, therefore, must be ascribed to the bran generally and not to adjacent endosperm or to the germ or any special region of the coat.

Experiments were then made to see whether the deteriorative effect could be counteracted in practice. Potassium bromate was found not to influence the effect. Although it produced improvement in the blend containing the groat meal, this was rather less than in the corresponding control. The point is illustrated by the following volumes of loaves from an untreated, rather weak 85% National flour, with and without the addition of 5% ground groats, and with and without the addition of 10 ppm potassium bromate.

	Untreated	Plus 10 ppm $KBrO_3$
Volume of control loaf (no groats)	2,827 cc	2,973 cc
Volume of loaf from 5% blend of ground groats	2,243 cc	2,316 cc
Percentage volume drop due to addition of ground groats	20.6%	22.1%

Even 1% of ground groats was found more than enough to annul the increase in volume of the control loaf resulting from 10 ppm bromate, although the crumb was rather closer-grained than the control (non-groaten) untreated loaf.

It was found that after a few months' storage in bags at 16° to 25°C, groats produced less deterioration when ground and added as a wholemeal to National flour. Storage of a mixture of wheat and groat flours at 14% moisture content in sealed tins at 25°C produced a marked change. In three months the deterioration due to the presence of the groats had disappeared.

Investigation showed that suitable heat-treatment of the groats was an effective and practicable means of counteracting the deteriorative effect of ground groats blended with wheat flour. Many experiments were made on (unground) groats in a horizontal rotating drum heated directly over a batswing gas burner. The temperature was measured by a thermometer inserted axially through the end of the drum. Its bulb was not in the grain and the readings are only a rough guide to air (and grain) temperature since no steps were taken to counteract bulb radiation effects. The groats were in effect heated in an atmosphere of partially saturated steam, because the only escape for the water vapor expelled from the grain was through a very narrow annular space around the thermometer stem. The influence of such treatment on the deteriorative effect of the wholemeal subsequently ground from the treated groats depended on the temperature and period of treatment as shown in Table II.

TABLE II

ANNULMENT BY HEAT TREATMENT OF GROATS OF THEIR DETERIORATIVE EFFECT ON BAKING QUALITY

(5% of ground groats mixed with 85% National wheat flour)

Temperature °C	Approximate decrease in deteriorative effect after heating for:							
	½ hr	1 hr	1½ hr	6 hr	8 hr	14 hr	48 hr	72 hr
100[1]	—	—	—	—	—	—	50%	50%
120[2]	—	—	—	—	30%	75%	—	—
140	25%	75%	100%[3]	—	—	—	—	—
150	—	100%[3]	—	—	—	—	—	—

[1] Progressive decrease from 8 to 48 hr at 100°C.
[2] Negligible decrease up to 6 hr at 120°C.
[3] Longer treatment darkens color and makes taste bitter.

The addition of 5% of wholemeal from groats treated for 1½ hours at 140°C had even a slight improving action on the National flour, the resulting loaf being larger and having a softer, better-developed crumb than the control. At the optimum treatment, 1½ to 2 hours at

140°C or 1 hour at 150°C, the taste is not adversely affected but there is a distinct deepening (browning) of crumb color.

The above treatment is not attainable in installations already existing on any commercial scale. Commercial oat-kilns do not afford oat temperatures above about 100°C.

The effect of direct steam treatment of groats has been investigated. It was found to be fairly satisfactory but not quite so good as that of the best "dry" heat treatment described above, chiefly in respect of loaf volume. Thus with 5% blends of ground groats with National 85% extraction flour, whereas the untreated groats caused a volume drop of over 20% as already described, the drop with the steamed groats was never more than 11% and in some cases less than this. Moreover, in all cases the steam treatment had an excellent effect on crumb quality.

The effect of the steam treatment was found: (*a*) to be uninfluenced by the extent of the incidental increase in moisture content of the groats, owing to condensation during steaming; (*b*) to be very little affected by the magnitude of the moisture content of the groats prior to steaming. It was only slightly better when applied to dried groats as against groats at their natural moisture content; (*c*) to be slightly favored by subsequent drying—because of the additional heat treatment involved, not because of the moisture removal *per se;* (*d*) to increase slightly but progressively with increase in period of steaming up to about 16 minutes. A 28-minute treatment was no better than a 16-minute except only that it produced a loaf of slightly finer texture; (*e*) to be rather favored by the use of superheated steam in a large-scale plant because the groats could be heated more quickly (with less condensation of moisture)—not because of any difference in temperature of treatment under these conditions.

In practice the steaming process might be preferable because, as described, possible variations in conditions make little difference to the result. The "dry" treatment would require a much closer degree of control and, moreover, produces slight changes in taste and color of the bread not found as a result of the steaming process. It is worth mentioning that rolled oats blend well into wheat flour at 5% dosage because in their preparation a preliminary heat treatment is followed, after dehulling, by steaming prior to rolling.

One way of stating the effective value (in wheat replacement) of the steaming process is that 5% of steam-treated groats, added as 100% wholemeal to National flour (of 85% extraction), would be equivalent in effect on baking quality to 4% of green groats in a grist affording 85% extraction flour.

It was found that the deteriorative effect of separated groat bran can be prevented by heat treatment of the bran itself. The treatment necessary is rather milder than that required with whole groats. Thus groat bran, in the raw state strongly deleterious to baking quality, was moistened to 19%, heated for 2½ hours at 120°C in a sealed tube, and tested in 2% and 5%[1] blends with National flour. The resulting loaves were substantially similar to the unblended control, the treatment having annulled the deleterious factor even more completely, particularly regarding loaf volume, than similar treatment of the whole groats.

Some experiments were made on the possibility of isolating and characterizing the nature of the injurious factor in groat bran. The following facts were ascertained, using as an index at each stage a baking test in which the volume and crumb characters of a loaf containing an appropriate proportion of the material under examination, introduced in part of the water used in dough making, were compared with those of the control. If the difference was equal to the deterioration with 5% of ground groats, the particular preparation was recorded as fully active.

1. The active agent is not fatty, since petroleum-ether-extracted groats and groat bran are fully active.

2. The agent is water-soluble and by repeated washing may be entirely removed from the bran so that the residue becomes inactive.

3. The agent is rather unstable in aqueous solution; its activity slowly decreases as the solution stands. Such aqueous solutions precipitate insoluble material.[2]

4. The agent is more readily extracted from the bran and preserved in solution by means of 3% salt solution. On dialyzing this extract to remove salt, a precipitate is thrown out which has only slight or no activity; substantially, the agent remains dissolved in the dialysate (A) with slight loss of activity. The agent appears either to accompany closely, or to form part of, the water-soluble protein of the bran. Thus:

5. It may be progressively precipitated from dialysate (A) by increasing concentration of ammonium sulfate above 60% saturation, or of alcohol. The earlier portions of this precipitate were much more active than the later, after redissolving and dialyzing. The proportion of solid matter in solution added in the baking test was in each case only 0.05% of the weight of the flour, but collectively (referred to later as Solution B) the two portions represented about 70% of the

[1] An addition of 5% bran would correspond, in terms of bran concentration, to an addition of about 20% whole groats.

[2] The work of Osborne (1891, 1891a, 1892) indicates that the proteins of the oat kernel undergo such changes with water or saline solution.

activity of the bran, the balance having been lost through instability during dialysis and through inclusion with the incidental precipitates and liquors. In both cases the solid matter contained 11 to 13% N and gave typically albuminoid precipitates when boiled in solution, this being much more marked in the solution from the earlier than the later precipitate.

6. The process of precipitation with ammonium sulfate, re-solution, and dialysis could be repeated—with a further partial loss of activity, probably through some denaturation during reprecipitation.

7. When either solution A or B was heated, it first became cloudy, then as boiling proceeded, threw down a flocculent precipitate. At the same time its activity progressively and markedly decreased—the decrease appearing to be *pari passu* with the precipitation.

It should, however, be noted that:

8. A similar process of extraction, dialysis, and precipitation carried out on heated bran, or bran milled from heat-treated groats, itself inactive, was found to give a visually similar series of solutions and precipitates, but the solutions corresponding to A and B above were inactive.

9. *Wheat* bran, whose soluble proteins afford a visually similar series of solutions and precipitates, shows no deteriorative effect on baking quality comparable with that of groat bran. Its dialyzed extract, in fact, was found to produce a slight improvement in loaf volume and crumb characters.

Shetlar and Lyman (1944) have reported that wheat bran contains a water-soluble improving factor, which considerably increases the volume of loaves baked from white flour doughs. They showed that, apart from this factor, wheat bran particles had a mechanical diluting effect on the baking properties of white flour to which they were added at the rate of 15%, like that of an inert substance such as cellophane. The deleterious nature of this mechanical effect was diminished on reducing the size of the added bran particles.

It should be mentioned here that the present authors found groat bran to be fully active in the peculiar way described only when ground fairly fine (to pass a 60 mesh screen). The unground bran in similar dosages produced a partial effect.

The question arose in discussion as to whether the deteriorative effect of groat bran could be due to the activity of amylases contained in it. Certainly there is some similarity in degree of stability on heat-treatment, but the authors consider that the effect of groat bran on bread is quite unlike that of sprouted grain, of malt, malt extracts, or of alpha-amylase. The point, however, was settled by measuring the alpha-amylase activity of two different samples of groat bran,

each of which showed fully the deteriorative influence on baking quality. The method used was that of Sandstedt, Kneen, and Blish (1939). Neither sample showed greater alpha-amylase activity than about 0.1 unit per g.

At this point the writers had to leave the subject. It is hoped, however, that this account of the properties and behavior of the water-soluble factor in oat bran so markedly antagonistic to baking quality may be of some interest to cereal chemists.

Summary

During the war, oats, in the form of green groats, have been used in the United Kingdom to replace a certain proportion of wheat. The nature of the groat is described and some analytical figures given. It is shown that a groat wholemeal (made by grinding 100% green groats to flour fineness) has a markedly depressing effect upon the baking quality of wheat flour. Even when only 2% is added to the latter, the loaf volume is reduced by about 15%. This is due to the groat bran, the addition of which in equivalent proportions substantially reproduces the deteriorative effect of the ground groats. This effect is only fully shown if the groat bran is ground fairly fine. It is not counteracted by potassium bromate but is diminished by storage either of the groats alone or of the blend of ground groats with wheat flour. It can be entirely counteracted by previous suitable heat-treatment of the groats, or of the groat bran only.

The deteriorative effect is due to a water-soluble agent which appears either to accompany closely, or to form part of, the water-soluble protein of the groat bran.

Literature Cited

Analytical Methods Committee
1943 Determination of the crude fibre in National flour. Analyst **68**: 276–278.
Jones, C. R., Simpson, A. G., and Kent, N. L.
1943 The addition of oats to National flour. Milling **100**: 230–233, 242–244, 261–263.
Kent-Jones, D. W.
1939 Modern cereal chemistry (3rd ed.), p. 458. The Northern Publishing Co. Ltd., Liverpool.
Moir, H. C.
1942 The nation's food. IV. Cereals as food. III. The chemical composition and nutritive value of oats and oatmeal. Chemistry & Industry **61**: 17–21.
Osborne, T. B.
1891 The proteids or albuminoids of the oat kernel. Am. Chem. J. **13**: 327–347.
1891a The proteids or albuminoids of the oat kernel. Am. Chem. J. **13**: 385–413.
1892 The proteids or albuminoids of the oat kernel. Am. Chem. J. **14**: 212–224.
Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.
Shetlar, M. R., and Lyman, J. F.
1944 Effect of bran on bread baking. Cereal Chem. **21**: 295–304.

EFFECT OF ROOM TEMPERATURE IN EXPERIMENTAL BAKING[1]

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Experimental baking is conducted over a wide range of climatic conditions which influence laboratory temperatures to varying degrees. Numerous expedients are used by baking chemists to overcome these temperature differences because it is well recognized that temperature produces significant effects upon fermentation. These various methods, such as cooling the mixer bowl, or warming or cooling certain of the baking ingredients, entail considerable trial and error with a consequent loss of time and possible increase in variability in baking results.

In recent years some laboratories, to offset the vagaries of the weather, have installed equipment for maintaining uniform conditions throughout the year. Such equipment was installed at Manhattan. Originally it was operated to maintain a room temperature of 77°F (25.0°C). In due course it was observed that many doughs came from the mixer below 86°F (30°C) which is stipulated for approved methods of the American Association of Cereal Chemists. To find out how room temperature influenced baking results, the experiments discussed in this paper were carried out some years ago.

As it is quite likely that air conditioning will become more prevalent in the future, it was thought that these results might prove helpful to those faced with the problem of deciding upon some definite room temperature under which to operate. Furthermore, it is hoped that these data may stimulate further work along similar lines, because the results reported can only strictly apply to a laboratory equipped similarly to that one in which the work was done.

Materials and Methods

Five bleached flours of pure varieties were available from millings made upon the 65-barrel commercial mill at Kansas State College. These flours were selected so as to provide a range in quantity and quality of protein. Table I gives the characteristics of these flours.

In the present series of baking tests the methods commonly followed in the laboratory were used excepting that the air temperatures in the baking laboratory were maintained at 87°, 82°, 77°, and 72°F

[1] Contribution No. 116, Department of Milling Industry.

TABLE I

CHARACTERISTICS OF FLOURS

Flour	Protein[1]	Ash[1]	Absorption	Mixing time[2]
	%	%	%	*Min.*
Comanche	14.6	0.44	66	3.2
Tenmarq (high protein)	14.0	0.41	64	2.9
Pawnee	13.7	0.41	64	2.0
Chiefkan	12.9	0.45	66	1.7
Tenmarq (low protein)	11.0	0.43	60	4.0

[1] 14% moisture basis.
[2] Mixing time for lean formula.

as required.[2] The fermentation cabinet was maintained at the customary 86°F (30°C). Punching and molding were carried out through the use of National sheeting rolls and a Thompson Moulder respectively. These mechanical aids as well as other equipment, flour, water, etc., were in equilibrium with the room temperature at the start of each day's bake, thus simulating conditions which would exist in a nonconditioned laboratory under different climatic conditions. Temperatures higher than 87°F (30.6°C) were not attempted because of the lack of adequate cooling facilities in the fermentation cabinet.

Doughs were mixed to the optimum in a Swanson-Working type mixer. Three-hour fermentations were used, punching after 100 and 155 minutes. Doughs were proofed for 55 minutes in low-form commercial-shape pans. Temperatures of all doughs were obtained after removal from the mixer, before the first punch, before the second punch, and before panning. Loaf volume was determined immediately after baking for 25 minutes at 425°F (218.3°C) in a Despatch electric oven with rotating shelf. Internal scores were determined the following day. These scores are relative only, with 100 representing the ideal.

Two formulas were used as follows:

Ingredient	"Lean" formula g	"Rich" formula g
Flour (85 g dry matter)	100	100
Yeast	3	2
Sugar	5	6
Salt	1	1.5
Dry milk solids	—	4
Malt syrup	0.3 (200°L)	0.25 (120°L)
Shortening (veg.)	—	3
Ammonium dihydrogen phosphate	0.1	—
Potassium bromate	0.001	0.003
Water (distilled)	as needed	as needed

In planning the experiment it was considered desirable to obtain data which could be analyzed statistically. The ideal, completely

[2] Temperatures of 87°, 82°, 77°, and 72°F correspond to 30.6°, 27.8°, 25.0°, and 22.2°C respectively.

randomized sampling, was not practical because the various room temperatures could not be changed within a day. However, the following plan was used: (a) all bakes at each temperature level were done during a given 3-day period; (b) during each day each flour in randomized order within each of the two formulas was baked five times; (c) both formulas were used each day, the order used being alternated. Thus 50 loaves were baked each day. Upon completion of the laboratory baking the data were brought together for variance analysis.

Results and Discussion

The experiment may logically be divided into two general phases: that pertaining to temperatures of the dough and that dealing with the baked bread.

TABLE II

MEAN DOUGH TEMPERATURES FOR ALL FLOURS AT INDICATED TIME DURING THE BAKING PROCEDURE

Room temperature in °F[1]							
87°F		82°F		77°F		72°F	
Lean	Rich	Lean	Rich	Lean	Rich	Lean	Rich
IMMEDIATELY AFTER MIXING[2]							
89.2	88.9	84.9	86.7	82.8	82.9	76.9	77.7
IMMEDIATELY BEFORE FIRST PUNCH[3]							
88.2	87.7	86.9	86.1	86.1	85.8	85.1	83.7
IMMEDIATELY BEFORE SECOND PUNCH[3]							
88.2	87.6	87.1	86.2	86.5	85.9	85.3	83.9
IMMEDIATELY BEFORE PANNING[3]							
88.0	87.7	87.0	86.3	86.3	85.7	84.6	83.8

[1] Room temperatures of 87°, 82°, 77°, and 72°F correspond to 30.6°, 27.8°, 25.0°, and 22.2°C respectively.
[2] 0.4°F is a significant difference, 0.5°F is highly significant.
[3] 0.1°F is a significant difference, 0.2°F is highly significant.

Dough Temperatures. As already mentioned, the temperature of each dough was taken after mixing, before each punching, and before panning. Table II gives the resulting mean temperatures obtained.

Examination of these data shows definite effects of room temperature upon the dough temperature in spite of a uniform fermentation

cabinet temperature. Different flours at a given room temperature tended to reach the same temperature when mixed to the optimum of each formula. Comparison of the data in the dough temperature table shows the greatest amount of temperature variation is at the mixing stage, the doughs tending gradually to approach 86°F (30°C) while in the fermentation cabinet. This is to be expected. However, dough is a poor conductor of heat, and unless it enters the fermentation cabinet reasonably close to the desired 86°F only part of the fermentation occurs at this required temperature. It may be observed that room temperatures of either 72°F or 87°F (22.2°C or 30.6°C) produced doughs which fermented either below or above the approved 86°F. Furthermore, on the average, a room temperature of 82°F (27.8°C) was too high for either formula, whereas 77°F (25.0°C) was too low for the rich formula.

Baking Results. It has been shown that room temperature influences the temperature of the fermenting dough. As temperature influences yeast development, it may be expected that these temperature differences will influence the finished bread. Table III gives the mean loaf volumes and Table IV the average internal scores of the baked bread.

TABLE III

MEAN LOAF VOLUME (CC) ACCORDING TO FLOUR, TEMPERATURE, AND FORMULA

Flour	Room temperature[1] and formula							
	87°F		82°F		77°F		72°F	
	Lean	Rich	Lean	Rich	Lean	Rich	Lean	Rich
	cc	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
Comanche	1069	1097	*1101*	*1105*	1021	1045	1001	1046
Tenmarq (high protein)	1116	1077	*1131*	*1078*	1060	1043	1035	1023
Pawnee	*1075*	1038	1067	*1048*	1013	999	984	969
Chiefkan	585	687	*636*	*695*	631	666	599	631
Tenmarq (low protein)	750	809	750	*831*	790	821	*797*	824

NOTE: Figures in italics are maximum volumes for formula.
[1] Room temperatures of 87°, 82°, 77°, and 72°F correspond to 30.6°, 27.8°, 25.0°, and 22.2°C respectively.

As anticipated, the widely different flours produced large differences in results. Room temperature also produced definite changes in loaf volume. The effect of formula was erratic and as shown later was found to be nonsignificant. It is interesting to note that, with two exceptions, maximum loaf volumes were obtained at a room temperature of 82°F (27.8°C). Apparently under the conditions existing in this experiment, maximum loaf volumes were favored by dough temperatures slightly over 86°F (30°C). Examination of these baking

data may prove helpful in coming to a decision as to operating room temperature in the baking laboratory. Final choice of a temperature will undoubtedly be influenced by particular techniques, equipment, and formulas used.

TABLE IV

AVERAGE CRUMB COLOR AND GRAIN-TEXTURE SCORES

	Room temperature[1] and formula							
	87°F		82°F		77°F		72°F	
	Lean	Rich	Lean	Rich	Lean	Rich	Lean	Rich
Crumb color[2]	83CY	89CW	83CW	85CW	84CW	86CW	85CW	88CW
Grain texture[3]	81–O	85–O	79–O	85–O	83–O	86–O	83–O	87–O

[1] Room temperatures of 87°, 82°, 77°, and 72°F correspond to 30.6°, 27.8°, 25.0°, and 22.2°C respectively.
[2] CY = creamy yellow; CW = creamy white.
[3] O = open.

The average internal loaf scores were not as consistent as either loaf volumes or dough temperatures and any interpretation is questionable. No attempt was made to study these values statistically. It is, however, evident that room temperature had little effect upon crumb color. The grain-texture scores, on the other hand, indicate best results come from cooler doughs. This may mean that the official 86°F (30°C) is somewhat high for production of the best bread.

Statistical Study

In the preceding discussion certain evident differences were brought out. The loaf volume and dough temperature data were subjected to variance analysis in order to determine the reliability of the conclusions reached by visual interpretation of the data. In addition it was hoped that possibly certain other relationships might be brought out by these statistical methods. Table V gives the variance analysis of the loaf volume data. It substantiates the observation that room temperature influences loaf volume significantly. Flour from the different wheat varieties produced very significant differences. The interactions of temperature × variety and formula × variety are highly significant. The nonsignificant interaction of temperature × formula probably indicates that the same *relative* loaf volumes were obtained by either formula regardless of the room temperature. Therefore, the same general conclusions on variety ranking would be obtained regardless of room temperature in so far as these particular samples are concerned.

TABLE V

ANALYSIS OF VARIANCE—EFFECT OF ROOM TEMPERATURE ON LOAF VOLUME

Source of variation	d.f.	M.S.
Temperature (T)	3	82,458*
Varieties (flour) (V)	4	4,379,140**
Formula (F)	1	37,921 n.s.
Replicate bakes	2	6,974 n.s.
Interaction:		
T × V	12	16,906**
T × F	3	735 n.s.
V × F	4	47,781**
Higher interaction	90	3,237**
Replicate error	480	1,097
Total	599	

n.s. Not significant.
* Significance exceeds 5% level.
** Significance exceeds 1% level.

Table VI gives the variance analysis of the dough temperatures. Room temperature was very significant in its effect. The interaction of time of reading with either formula or with room temperature was very significant. The interaction of formula × time of reading may be explained as a change in temperature differential between the two formulas as the room temperature changes.

TABLE VI

ANALYSIS OF VARIANCE—EFFECT OF ROOM TEMPERATURE ON DOUGH TEMPERATURE

Source of variation	d.f.	M.S.
Formula (F)	1	109.23 (n.s. by F × TR)
Temperature (T)	3	3,200.18**
Time of reading (TR)	3	899.38 (n.s. by T × TR)
Varieties (flour) (V)	4	4.19 n.s.
Replicate bakes	2	33.87**
Interactions:		
F × T	3	6.47 n.s.
F × TR	3	65.06**
F × V	4	.49 n.s.
T × TR	9	451.00**
T × V	12	.97 n.s.
TR × V	12	3.58 n.s.
Other interactions	423	2.63**
Replicate error	1920	.66
Total	2399	

n.s. Not significant.
** Significance exceeds 1% level.

Summary

To study the effect of room temperature upon baking results, four different room temperatures, two formulas, and five flours were used. Fifteen replicate bakes were made for each combination.

Immediately after mixing, before the first and second punches, and before panning the doughs, dough temperatures were taken during the production of the resulting 600 loaves.

Analysis of the data showed that changing the room temperature influenced both dough temperatures and loaf volumes significantly.

Over the 15°F (8.4°C) range used in this study the different flours tended to maintain the same relative rank in loaf volume at all temperatures. This indicates that maintenance of a constant room temperature is of more importance than any specific temperature within the range of temperature studied.

A room temperature of approximately 82°F (27.8°C) favored maximum loaf volume, but superior grain-texture scores resulted from lower room temperatures.

REPORT OF THE 1944–45 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

FRANK R. SCHWAIN, *Chairman*

Kroger Grocery and Baking Company, Columbus, Ohio

(Received for publication August 3, 1945)

For several years reports have appeared in the literature suggesting that granulation may influence the spreading properties of a cooky flour (Loving, 1942; Hanson, 1943, 1943a; Schwain, 1944). On the basis of laboratory tests, coarsely ground flours exhibited better spread in cooky tests than finely ground flours (Hanson, 1943, 1943a). A semicommercial test, however, favored the finely ground flour in a wire-cut study (Hanson, 1943a).

The committee's tentative test cooky formula has shown satisfactory replication between laboratories and, on the basis of last year's work, appeared to be adaptable for selecting flours particularly adapted to the production of wire-cut or sheeter goods. These findings were based on the correlation of laboratory data with actual shop results (Schwain, 1944).

With this background the committee this year undertook a study of two flours differing primarily in particle size. Four laboratories tested the flours and four bakeries ran commercial tests.

Materials and Methods

The laboratory cooky-baking tests were conducted in two ways. The first method, originally suggested by Hanson (1943), was the one employed by previous committees. The second method was proposed

by P. W. Hodler (a member of the present committee) and represented an attempt to obtain more uniform dough consistency by controlling the amount of dry matter and the quantity of moisture added.

Two unbleached straight grade flours milled from the same lot of Michigan white wheat, but differing in granulation, were employed in

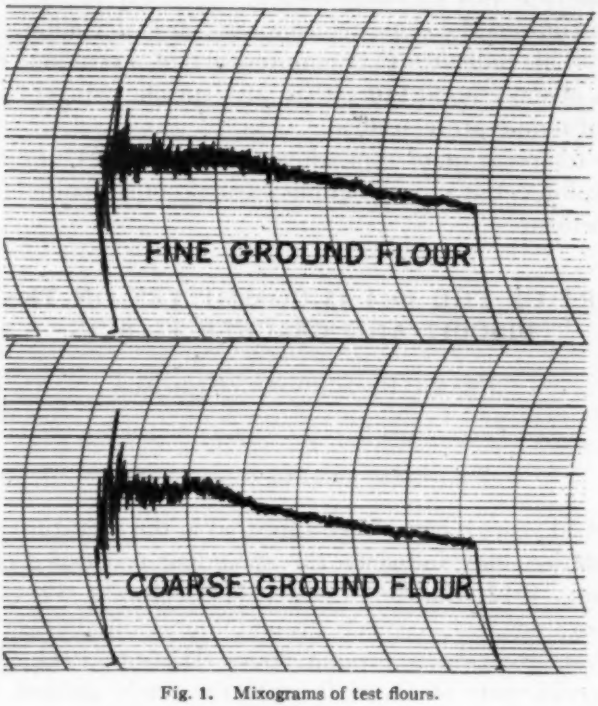

Fig. 1. Mixograms of test flours.

TABLE I

ANALYSES OF TEST FLOURS

Flours	Ash[1]	Protein[1]	Gluten		Maltose value	Apparent viscosity	
			Wet	Dry		No-time	1 hour
	%	%	%	%	*mg*	*Degrees MacMich.*	*Degrees MacMich.*
Coarse	0.43	7.8	24.6	8.8	128	20	34
Fine	0.42	7.9	24.9	9.1	131	24	42

[1] 14% moisture basis.

the studies. The differences in granulation were produced by changes in the grinding and bolting operations at the mill. The analyses of the flours are recorded in Table I and the granulation data in Table II. Mixograms made with the flours are reproduced in Figure 1. These results show that, except for granulation, the flours are very similar.

TABLE II

GRANULATION OF TEST FLOURS [1]
(Percent of total flour)

	Collaborator 2		Collaborator 9	
	Coarse	Fine	Coarse	Fine
	%	%	%	%
Over 11 XX silk	0.0	0.0	2.5	0.0
Over 12 XX silk	11.0	1.9	2.2	0.5
Over 13 XX silk	16.8	5.3	6.5	2.5
Over 14 XX silk	13.3	10.5	12.0	10.5
Over 15 XX silk	17.0	24.4	15.3	28.2
Through 15 XX silk	41.9	58.0	61.5	58.3

[1] Flours were bolted for 30 minutes by means of a Ro-Tap shaker.

The cooky-test procedure of Hanson (1943) employs 224 g of flour (13.5% moisture basis) and 19.5 ml of distilled water. The method proposed by Hodler requires 225 g of flour (14.0% moisture basis) and 16 ml of distilled water; where the moisture content of the flour differs from this standard, the amount of added water is adjusted to give the same total moisture (47.5 ml) in the dough.

Results

The results obtained by the collaborators with the methods of Hanson and of Hodler, designated as "standard absorption" and "adjusted absorption" respectively, are recorded in Table III. When

TABLE III

LABORATORY SPREAD FACTORS (*W/T*) OF COOKIES MADE AT DIFFERENT ABSORPTIONS FROM FLOURS VARYING IN GRANULATION

Flour	Collaborator								General average	
	1		2		3		4			
	Std. abs.	Adj. abs.	Std. abs.	Adj. abs.	Std. abs.	Adj. abs.	Std. abs.	Adj. abs.	Std. abs.	Adj. abs.
Coarse	9.45	9.65	8.83	8.33	9.25	9.50	8.42	9.00	8.99	8.87
Fine	9.50	9.17	7.87	7.54	8.50	8.80	8.12	8.18	8.49	8.42

the dough consistency was regulated by adjusting the absorption, all collaborators ranked the flours in the same order and the differences in *W/T* only varied from 0.82 to 0.48 for the different collaborators. In contrast, the regular method, employing the "standard absorption," gave differences in *W/T* between the two flours which varied from 1.00 to − 0.05 for the different collaborators. It is noteworthy that

these laboratory cooky tests showed that the coarsely ground flour had better spreading properties than the finely ground flour, thus confirming previous laboratory findings.

The results of regular shop tests with the two flours, carried out in commercial shops, are recorded in Table IV.

TABLE IV

SPREAD FACTORS (W/T) FOR COMMERCIAL TESTS [1]

Flour	Collaborator							
	5	6		7	8			General average
WIRE-CUT COOKIES								
Coarse	7.60	7.01	7.86[2]	7.67	7.70		6.25	7.25
Fine	7.70	7.19	7.64[2]	7.92	7.89		6.64	7.47
ROTARY PIECES								
Coarse		6.45		7.37	7.51	10.97	9.10	8.28
Fine		6.09		7.33	7.31	10.78	9.10	8.12
SHEETER GOODS								
Coarse						8.99		
Fine						8.97		
BAR GOODS								
Coarse	3.06							
Fine	2.82							

[1] Where the collaborator tried several kinds of wire-cut or rotary pieces, the results are listed side by side.

[2] Results not averaged since this cooky was not a pure type.

With wire-cut goods the finely ground flour in almost all cases outranked the coarsely ground flour. This is in direct contrast to the laboratory predictions. The grain and symmetry of cookies made with the finely granulated flour were also preferred by the bakeries. It would appear that the laboratory test is sensitive to flour granulation. For rotary goods, the coarsely granulated flour was superior, that is, the results were in line with the laboratory tests. Little or no advantage of one flour over another was shown in regard to the tendency of the cookies to check.

It was conjectured that the differences between the performance of the flours in the laboratory and in the shop might be explained by differences in the extent of mixing. With wire-cut doughs, mixing is only carried out to clear the machine, thus permitting only a minimum

of development. With the laboratory test, mixing is a fixed factor and the more finely ground flour, which tends to hydrate more quickly, could conceivably undergo some development, and hence show less spreading. Rotary doughs, on the other hand, are mixed more and at a lower absorption, all of which contributes to toughness. A finely ground flour tending to develop faster would probably yield cookies that would spread less. These suggestions are in line with the results of the laboratory and shop tests.

The collaborating laboratories were accordingly asked to repeat their tests in two ways. One investigator was asked to use the regular laboratory test formula but to reduce the mixing by 50%, making only a one-minute mix in the last stage. Two other collaborators were asked to employ one of the regular commercial wire-cut cooky formulas (a sugar type cooky) on a laboratory scale and also to reduce the mixing in the last stage (after the flour is added) from two minutes to one minute. The results are recorded in Table V.

TABLE V

COMPARISON OF *W/T* FACTORS FOR COOKIES MADE WITH SHOP AND LABORATORY FORMULAS

Bakery procedure		Collaborator No.	Spread factor (*W/T*)	
Formula	Test		Coarsely granulated flour	Finely granulated flour
Commercial	Commercial	8	7.70	7.89
Commercial	Laboratory (2-min. final mix)	1	9.55	8.88
		2	9.81	9.46
Commercial	Laboratory (1-min. final mix)	1	8.80	8.85
		2	9.31	9.24
Laboratory	(2-min. final mix)	4	8.42	8.12
Laboratory	(1-min. final mix)	4	8.78	8.40

With the laboratory formula, reduced mixing increased the spread of cookies made from both flours but failed to change the ranking of the coarse over the fine flour. This agrees with the findings of Loving (1942). The commercial formula, when used in the laboratory, ranked the coarse flour much better than the fine with a two-minute mix; however, when the mixing was reduced to one minute, the fine flour performed on a par with the coarse. This is somewhat more in harmony with shop practice and bears further investigation. Time did not permit further experimentation but it would seem that even shorter mixing times with this formula should be explored. The

problem appears to be one of formulation and mixing in order to make laboratory size batches perform in a similar manner to those prepared under commercial conditions.

Conclusions and Recommendations

Finely ground flour appears to have some advantages over a more coarsely granulated product in the commercial production of wire-cut goods. For the production of rotary type goods, however, a more coarsely granulated flour, which tends to develop more slowly, is preferable; such a flour tends to give less toughness in the dough and yields cookies exhibiting better spread.

The current laboratory test is sensitive to flour granulation and gives an unsatisfactory estimate of flour quality for the production of wire-cut goods.

It is fortunate that this weakness was uncovered before it was proposed as an official test. In the committee's opinion an official formula must satisfy at least three criteria: (1) ease of replication; (2) ease of differentiation of flour types; and (3) accurate forecast of shop performance. This third condition is probably the most important since fine distinctions between flours which are not borne out by performance in industry are of little practical value.

It is suggested that work be undertaken next year to overcome this weakness in the test. A critical examination of the formula should be made in an effort to bring the results of laboratory tests into line with shop performance. The use of a typical commercial formula with variations in mixing should prove to be a most promising method of attack.

Acknowledgments

The chairman expresses appreciation to the entire committee for willingness to undertake even more assignments than were originally planned. Members of the committee were: Miss Pearl Brown, W. H. Hanson, T. E. Hollingshead, P. W. Hodler, Jan Micka, H. M. Simmons, O. P. Skaer, and L. S. Thomson. Acknowledgment of industry's contributions are due Henkel Flour Mills, for the test flours, and Perfection Biscuit Company, Kroger Grocery and Baking Company (Columbus Unit), and the Sawyer and Strietmann Divisions of the United Biscuit Company for their commercial tests. Special thanks are due Clifford Nelson for the mixogram study.

Literature Cited

Hanson, W. H.
1943 Report of the 1941–42 committee on testing biscuit and cracker flours. Cereal Chem. **20**: 314–318.
1943a Report of the 1942–43 committee on testing biscuit and cracker flours. Cereal Chem. **20**: 595–598.
Loving, H. J.
1942 Report of the 1940–41 committee on testing biscuit and cracker flours. Cereal Chem. **19**: 358–364.
Schwain, F. R.
1944 Report of the 1943–44 committee on testing biscuit and cracker flours. Cereal Chem. **21**: 407–412.

COMMUNICATION TO THE EDITOR

A Simple Test for Riboflavin in Enriched Flours

Received June 5, 1945

SIR:

A water extraction coupled with visual examination of the resulting supernatant liquid under ultraviolet light in comparison with similar extracts of flours of known riboflavin content has been found to provide a reliable indication of the total riboflavin content of enriched flours.

Rapidity and ease of manipulation of the test make it valuable for the rapid examination of large numbers of samples without the use of expensive equipment. It is well adapted to routine mill control practice, and a highly skilled operator is not needed for accurate estimation.

A flour suspension is formed by weighing 10 g of the unknown sample into a 500 ml Erlenmeyer flask. One hundred ml of distilled water are added, a rubber stopper is inserted, and the flask shaken vigorously until all flour is thoroughly wetted. The flour is extracted at room temperature for 30 minutes, shaking vigorously at the end of each 10 minutes. At the end of 30 minutes the flask is shaken vigorously and part of the sample is immediately transferred into a pyrex test tube. The suspension is then allowed to settle for 20 minutes, or centrifuged if a centrifuge is available.

Reference standards are prepared in exactly the same manner as outlined for the unknown sample. Extracts of the sample and reference standards are prepared at the same time and this eliminates the necessity of working entirely in the dark since light destruction of riboflavin is of the same magnitude in both the unknown samples and the reference standards. Care should be taken, however, to avoid exposing unknown samples or standards to strong light. The reference standards can be made using any increments of riboflavin content desired. Each of the following procedures has proved satisfactory.

Either secure a nonenriched flour of known riboflavin content and add definite increments of riboflavin or, using a simpler procedure, secure an enriched flour of known analysis and dilute stepwise with nonenriched flour. Standard flours varying by 0.1 mg/lb over the range of 0.7 mg/lb to 1.5 mg/lb are sufficient to cover the ranges encountered in enriched flours. Within these limits the natural riboflavin of the diluent flour may be disregarded since it is thought to be incompletely extracted and hence introduces a negligible error. These standard flours may be stored in tightly closed dark bottles for at least 3 months without serious deterioration.

Comparison of the extract of the unknown sample with extracts of the reference standards is made in a dark room with a strong ultraviolet light such as that produced by the H-4 Black Lamp, manufactured by Continental Lithographing Company, Cleveland, Ohio. This light source is improved by the use of Corning filter, Red Purple Corex No. 9863.

Analysis of 39 samples by this method and our regular fluorometric procedure gave a correlation of + .90 between the two methods. These tests were made over a period of several months and represent the work of three different operators. When operators have acquired some experience in applying the test, day-to-day reproducibility of the test is good and concordant results between operators are attainable.

PAUL W. HODLER and GEORGE GARNATZ
Kroger Food Foundation, Cincinnati 4, Ohio

BOOK REVIEW

Industrial Oil and Fat Products. By Alton E. Bailey. 735 pp. Interscience Publishers, Inc., New York, N. Y. 1945. Price $10.00.

This book is intended primarily as a text on oil and fat technology. Consequently, the major emphasis is placed upon a description and discussion of the commercially important oil and fat products and the processes used in their manufacture. In addition, two preliminary sections offer a succinct review of the chemical and physical nature of fats and oils, as well as a consideration of their composition, characteristics, and availability. Throughout the book, the author has tied in theory with practice and wherever possible has attempted to keep the discussion on a quantitative rather than a qualitative basis.

The first four chapters (83 pp.) deal with the structure and composition of fats and oils, chemical and physical properties of fats and fatty acids, and the role of fats in the diet of man. The next four chapters (106 pp.) are concerned with the sources, utilization, and classification of fats and oils, their production and consumption, and the composition and characteristics of the individual members. This is followed by the first major section of seven chapters (222 pp.) taking up cooking and salad oils; salad dressings; plastic shortening agents; butter and margarine; bakery products and confections; soap and other surface-active materials; paints, varnishes, and related products; and miscellaneous oil and fat products. The second major section of nine chapters (300 pp.) proceeds with a discussion of the extraction of fats and oils; refining and bleaching; deodorization; hydrogenation; soap making; fractionation of fats and fatty acids; fat splitting, esterification, and interesterification; polymerization, isomerization, and related processes; and solidification, homogenization, and emulsification.

Throughout the book the author has done an excellent job of critically surveying existing literature and in addition has presented a considerable amount of previously unpublished technical material arising from his own experience and that of many friends in the industry. The literature references are as up-to-date as is possible in a book of this size written by a single author. Furthermore, the placing of references at the foot of the page rather than at the end of each chapter greatly facilitates their use.

Dr. Bailey's book fulfills a long felt need in the industry and should prove very useful to any chemist or technologist dealing with industrial fats and oils.

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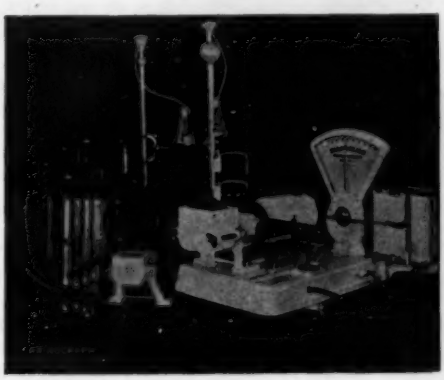

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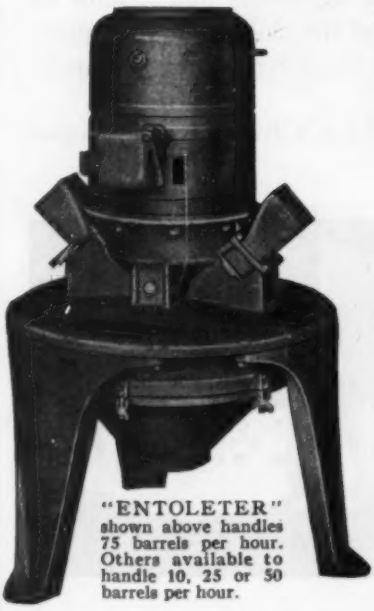

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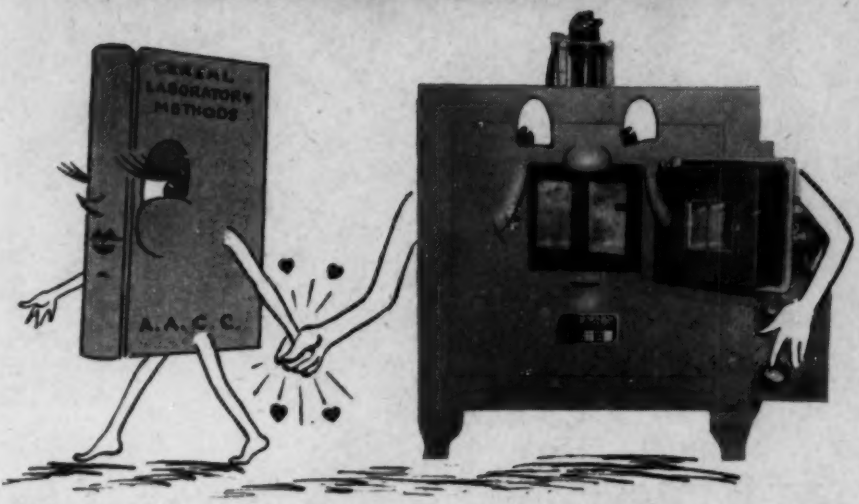

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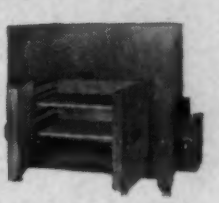

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Thomas Burr Osborne Medal

to

John C. Baker

at a special meeting of the

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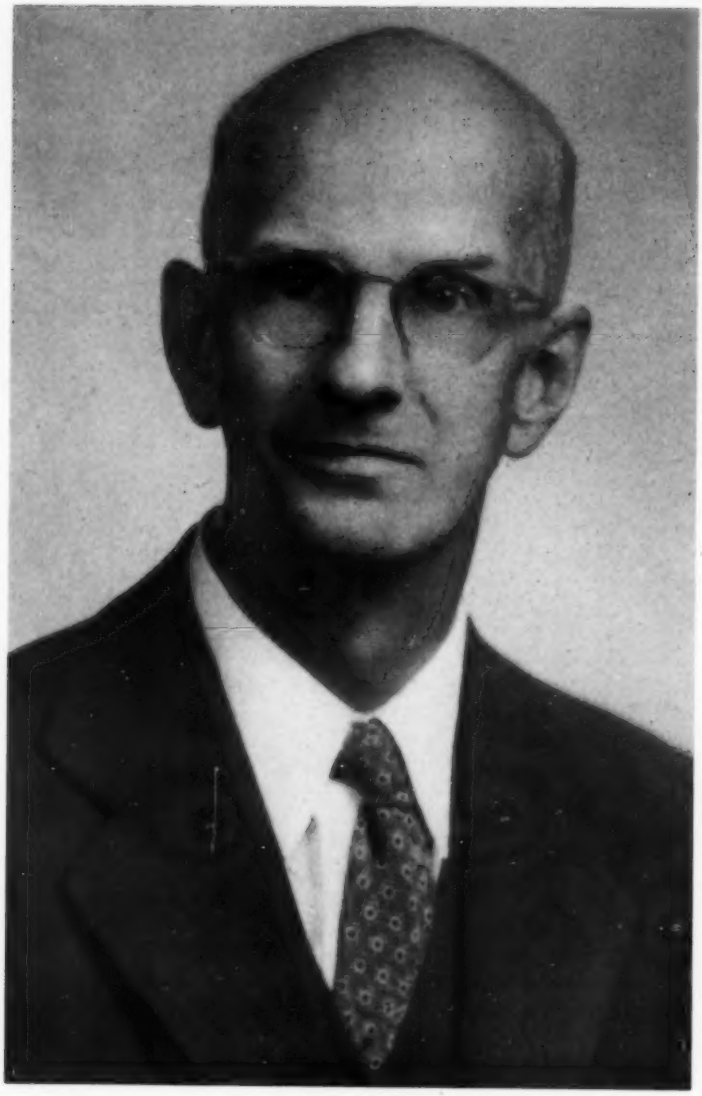
of the

American Association of Cereal Chemists

Hotel New Yorker

May 15, 1945

The banquet was preceded by a reception in honor of Dr. Baker, given by C. F. Wallace and M. F. Tiernan of the Wallace and Tiernan Company, Inc., where Dr. Baker spent his most productive years of research and where the scientific investigations leading to the award of the medal were carried out. Approximately two hundred people attended the reception and the banquet which followed.



JOHN C. BAKER

INTRODUCTORY REMARKS OF THE CHAIRMAN OF THE NEW YORK SECTION, AMERICAN ASSOCIATION OF CEREAL CHEMISTS

J. H. KARRH

Ladies, members of American Association of Cereal Chemists, guests, and friends of John C. Baker:

As I gaze upon those present at this gathering, I am impressed with the large number of out-of-town members and it reminds me of a gathering similar to that of the national convention. The New York Section is honored to have you with us this evening and I am pleased to be privileged to extend to you a most hearty welcome.

The national war effort has bestowed hardships on us right and left, but to my knowledge this is the only time that the hardships of the war effort have brought anything good; for with the cancellation of the national convention, the New York Section has the honor of acting as host to the Osborne Medal Award Committee and is enjoying the ceremony that is usually the privilege of the national convention. We feel doubly honored on this occasion as the recipient of the Osborne Medal Award is one of our local group.

The friends, relatives, and co-workers of the recipient of this award naturally are proud and glad to do honor to his achievements. However, none of us can reach the depth of joy that must be in the heart of the individual who, after so many years of effort, can feel that his being on this earth has made it a better place to live. One only has to look back on the record of the recipient of this award to know that he has made this a better place to live in and we as fellow members of the New York Section as well as members of the national Association are proud of Dr. John C. Baker.

I could go on and recite many laudable achievements of Dr. John C. Baker but more capable minds are waiting for this privilege and it gives me great pleasure to introduce, at this time, the master of ceremonies, Dr. Charles G. Ferrari, chairman of the Osborne Medal Award Committee.

THE THOMAS BURR OSBORNE MEDAL

REMARKS BY THE CHAIRMAN OF THE OSBORNE MEDAL AWARD COMMITTEE, CHARLES G. FERRARI

Mr. Karrh, members of the American Association of Cereal Chemists, and guests:

It is very gratifying that such a large group has gathered here this evening to honor the man for whom this meeting is being held.

To satisfy the curiosity that some of you may have respecting the identity of the people at the speakers' table, I will name them, beginning on my right. I will not ask them to rise. Mr. Paul Logue, Mrs. C. G. Ferrari, Mr. George Kirby, Mrs. Oscar Skovholt, Mr. John Karrh, Mrs. John C. Baker, the wife of the medalist (will you please rise, Mrs. Baker), Dr. John C. Baker, the medalist, Dr. J. Avery Dunn, Mrs. John Karrh, Dr. Oscar Skovholt, Mrs. George Kirby, Mr. John C. Baker, Jr. (will you please rise, Mr. Baker), Mrs. J. Avery Dunn, and Mr. G. Dalby.

There are five past presidents of our national Association in the audience. I will name them and the year in which they were president, and ask them to rise:

Leslie Olsen	1928
R. C. Sherwood	1934
George Garnatz	1940
Claude Davis	1941
C. N. Frey	1942

The purpose of our meeting tonight is to present the Thomas Burr Osborne gold medal of the American Association of Cereal Chemists to John C. Baker. He was the unanimous choice of the Osborne Medal Award Committee, which consisted of the following members: Dr. C. H. Bailey, Dr. M. J. Blish, Dr. J. Avery Dunn, Dr. Ralph K. Larmour, Dr. Charles O. Swanson, and myself as chairman. The action of the committee had the unanimous endorsement of the Executive Committee.

The medal has been presented heretofore at an annual meeting of the national Association. Because our annual meeting—in common

with many others—was cancelled as a war measure, we are meeting tonight under the auspices of the New York Section. It is fitting that we do so, because the New York Section is a large and virile group and Dr. Baker has always been an active member of it.

On behalf of the national officers, the Osborne Medal Award Committee, and members everywhere I wish to acknowledge this courtesy and thank the officers and members of the New York Section for making the present medal award ceremonies possible.

The reception which preceded this dinner—given by Mr. C. F. Wallace and Mr. M. F. Tiernan, of the Wallace and Tiernan Company, Inc., in honor of their friend and associate Dr. Baker—has contributed much to the enjoyment of this occasion. They have provided the music and are responsible for the gardenias which adorn the ladies and the white carnations which the men are wearing. In more ways than one, white flours seem to be an old tradition with them. We are happy to have them participate with us in making this a memorable event, and welcome those associates of Dr. Baker who found it possible to be here tonight.

The plan of the medal award was conceived in 1926—and in 1928 the first gold medal was presented to Thomas Burr Osborne, in whose honor the medal was named. The Osborne medal may be conferred for distinguished contributions in cereal chemistry or in a closely related scientific field. While the medalists to date have been United States citizens, there is no limitation in that respect, and the recipient need not be a member of the American Association of Cereal Chemists.

Thomas Burr Osborne distinguished himself by fundamental contributions to the knowledge of cereal proteins. Many of our modern concepts of nutrition had their beginning in his classical experiments made in the early nineteen hundreds. He suggested that there must be a significant difference between the nutritive value of proteins having widely different amino acid compositions. To test this point he prepared purified proteins and fed them singly and in combination with other proteins or individual amino acids. He showed that malt amylase was protein in nature. He pioneered in the field of vitamins and is regarded as a co-discoverer of vitamin A.

The second Osborne medal was awarded in 1932 to Clyde H. Bailey—the dean of cereal chemistry in its modern sense. His contributions covered an extremely wide range of interests. He used and forged new scientific tools and procedures with which he added greatly to the quantitative aspects of cereal chemistry. He became

famous as a teacher and attracted students from all over the world. He has published voluminously, has written two monographs, founded the journal of *Cereal Chemistry*, and has served the Association as its president. His, indeed, has been a distinguished career.

The third Osborne medal was awarded to Morris J. Blish in 1936. He contributed to our knowledge of the chemical constitution of cereal proteins, devised a direct method for the determination of glutenin, studied critically the effect of frost damage on wheat, added to our knowledge of the diastatic activity of flour, and was chiefly responsible for changing the experimental baking procedure from an art to a scientific test. Many and stormy were the debates on the floor of our annual meetings before this was accomplished. He too served our Association as president and for a long time was editor of *Cereal Chemistry*.

Charles O. Swanson was awarded the fourth Osborne medal in 1938. He achieved fame as a scientific worker, a teacher, and a writer. He studied the bases of flour quality, measured the influence of various agronomic factors on the quality of wheat, and studied extensively the colloidal properties of wheat flour doughs.

Ross Aiken Gortner was awarded the fifth Osborne medal in 1942. He was a great teacher and an imaginative research worker. He applied newly formed concepts of colloid and physical chemistry to the study of cereal proteins, wheat flours, and doughs. His studies on the chemistry of the proteins founded a school of thought to which his name is frequently attached. His publications were voluminous and his book entitled *Outlines of Biochemistry* is a treasurehouse of information.

There is no prescribed periodicity for the Osborne Medal Award and it has not been conferred frequently. Since its founding in 1926 there have been only five medalists.

The purpose of reviewing the nature of the award, and describing briefly the scientific achievements of former medalists, has been to lend emphasis to this—that John C. Baker tonight will join a distinguished company of scientists who have been similarly honored: Osborne, Bailey, Blish, Swanson, and Gortner.

The next speaker will dwell at greater length on the achievements of the present medalist. Dr. J. Avery Dunn, a member of the Osborne Medal Award Committee, was privileged to work with Dr. Baker some years back. He will tell us about John C. Baker, the man and the scientist. Dr. Dunn.

JOHN C. BAKER—MAN AND SCIENTIST

J. AVERY DUNN

There are several reasons why this is a memorable occasion. We are living in an era of our national history that has been characterized by the breaking of precedents. In fact, it almost seems that breaking of a precedent is the rule and keeping is the exception. This occasion is also in step with the times in that tonight precedents are being broken. Because of the effect of the war upon our transportation facilities, the Osborne medal is being presented at a dinner meeting sponsored by a local section of the American Association of Cereal Chemists rather than at a dinner which is an integral part of an annual meeting of the national Association. Furthermore, we are tonight conferring this honor upon one of our members who has distinguished himself in the *industrial* application of cereal chemistry, whereas all former recipients received the medal for outstanding achievements in the *academic* application. To the list of Osborne medalists, which up to the moment contains the names of such celebrated scholars as Thomas Burr Osborne, C. H. Bailey, M. J. Blish, C. O. Swanson, and R. A. Gortner, we are now about to add the name of John C. Baker, who early in his career migrated from pedagogy to industry.

Our particular branch of science, called cereal chemistry, is a relatively new twig on the chemical branch of the scientific tree, and doubtless this accounts for the stress we have placed during the past three decades on the value of fundamental academic research and upon teaching, since it is largely through the teaching of scientific fundamentals that a virile branch will grow. Tonight, after thirty odd years of sound growth and development, we see evidence indicating that we are, indeed, coming of age.

May I quote from the "foreword" by our past president, Dr. Betty Sullivan, which was published in the program of our thirtieth annual meeting: "Scientific research by industry need not have for its sole objective immediate practical value even during the war, for time spent on theoretical projects today may hasten victory and lead to future economic gain. *But let us always look for the use of every fact we find* and, whether we work on theoretical or practical problems,

we must still serve the Truth, since the search for Truth insures progress and the knowledge of Truth constitutes our ultimate happiness." Tonight's medalist has, indeed, always looked for the use of every fact he discovered and those of us who have been associated with him can vouch for his diligence in searching for the Truth.

A moment's thought will show that other scientific groups have gone through essentially the same history as have we. Eventually, with the scientific branch in a healthy state of growth, recognition in the form of a medal has been accorded such industrial researchers as Steinmetz, Langmuir, Kettering, Bakeland, and Midgeley, whose scientific contributions have served mankind in a very practical fashion. In conferring the Osborne medal on John C. Baker, whose contributions to the field of cereal chemistry have been intensely practical, mechanically ingenious, and of outstanding service to mankind, our Association is, indeed, in excellent company.

Tonight's medalist was born in Ridgeway, Iowa, on August 11, 1884. He received his undergraduate training at Upper Iowa University, receiving the degree of Bachelor of Science in 1910 and an honorary degree of Doctor of Science in 1933. He was an assistant on the staff of Upper Iowa University for a short time in 1910 and for the next three years was head of the Department of Science at New Mexico Normal University. It is noteworthy that at this point —early in his life—he made two decisions, both of which have influenced his career very greatly. First, he realized that he needed graduate training and, although he was married at the time, he left his teaching position for an assistantship at Columbia University, receiving the Master of Arts degree in 1915 and the doctorate in 1916. The second decision was his choice of an industrial career from that date—forsaking the teaching profession.

Three fruitful years were spent as an associate chemist at the New York State Agricultural Experiment Station at Geneva, New York, with Dr. L. L. Van Slyke. From 1919 to the present time, Dr. Baker has been associated with Wallace and Tiernan Company, Inc. At the outset of his industrial career, he was chief chemist. Today, he is director of research of Wallace and Tiernan and the associated companies. He is vice president and director of Wallace and Tiernan Products Company, Inc., and of the Sales Corporation, and he is a director of Novadel Agene Corporation, Novadel Agene, Ltd., Lucidol Corporation, and the Industrial Appliance Company. Incidentally, Wallace and Tiernan Products Company, Inc., and

Novadel Agene Corporation are companies originally organized around his inventions. One of the most important of these was the development of flour-bleaching and maturing methods employing nitrogen trichloride. This method is known throughout the world as the Agene process. It has revolutionized flour bleaching and maturing and, economically at least, is, in the opinion of many, the most important contribution of the science of cereal chemistry to mankind. I question that any development in the field of flour milling since the introduction of the middling's purifier has had a more far-reaching effect upon its operations.

Prior to the introduction of the Agene process, it was necessary, as we all know, to store freshly milled flour for several months in order that it might have an opportunity to mature through natural processes. Although the introduction of instantaneous maturing was, in the light of our present knowledge, a "natural" for the milling industry and the means of eliminating a slow and very costly aging period, it was not an easy task to convince flour millers of its desirability, particularly in the early days. Today, however, there are 552 installations of the Agene process in the United States and Canada, and 259 installations in Great Britain. It is estimated that in 1944 over 91,000,000 barrels of flour were treated with the Agene process. The cost of storing this quantity of flour for even sixty days is staggering in itself in normal times, but in time of war one must also consider the manpower necessary to handle flour in storage and the possibility that losses may occur through insect infestation, fire, and flood. Surely this process has contributed materially to the progress of the milling and baking industries. Further discussion, particularly before a group of cereal chemists, seems futile, indeed.

Dr. Baker has been interested in a rather wide field. In all, he has published thirty-two scientific papers and has been granted twenty-nine U. S. patents, in addition to a number of patents which he holds in Great Britain, Canada, and Germany. His papers cover the fields of enzymes, proteins, milk, textiles, sewage, water purification, and condenser de-sliming processes.

German composers of the classical school followed a practice calculated to leave no doubt in the minds of music students as to the real merit of their compositions. Accordingly, it was their custom to label an opus as "berühmt" for the most part, but occasionally as "nicht so berühmt." Apparently their works were all either famous or close to it and none, I am sure, were ever infamous. By devious

and diverse means, I have succeeded in having Dr. Baker so rate his contributions and it is interesting to learn that, in his opinion, outside of the papers relating to flour, "not many are important." He would have considerable trouble in convincing many of us that such is the case. Although his earlier work with alpha and beta amyloses and amylases, as well as his work on casein, was interesting and important, his three papers on a simple method of testing for mastitis in cows were of great importance to the dairy industry and have been quoted widely. This method permitted a quick detection and isolation of infected cows.

Dr. Baker's patent covering the use of a limestone tower in neutralizing a solution of chlorine in water is today a widely used process. His discovery of the de-sliming process has done much to speed the day of victory, for there are nearly 500 installations of slime-control equipment in operation in this country, and one finds them in plants where steel, smokeless powder, TNT, synthetic rubber, aviation gasoline, and industrial alcohol are made, and power plants without number make use of this method. The saving in coal through increased efficiency is approximately 2.5 percent.

The development of a residual chlorinator is another interesting contribution. This equipment controls the amount of chlorine applied to water by the amount present in the water after application and thus corrects for the chlorine demand of the water as well as for the variation in flow.

Another process, in which nitrogen trichloride is used in high dilution with air, is known as the Decco process. The application of this gaseous mixture in the handling of fruit and vegetables in order to kill mold organisms and thus retard decay is finding wide use, principally in California, not only in the handling of oranges, lemons, and grapefruit, but also tomatoes and cantaloupes.

A patent covering the making of bread has been held by the patent-office courts to be the first patent application and disclosure of the whitening of bread by small amounts of soya-bean flour. Prior claims have recently been allowed to this patent after much litigation.

Other developments cover the use of chloramines, the development of a colorimeter, an apparatus for the therapeutic application of chlorine, a shortening compound, a method of making laundry bleach, a method of improving the taste and odor of water, the use of a silica-gel protector in chlorination, the use of fused silver chloride for joining glass to metals, an electrolytic cell, a method of testing dough,

a rubber printing pad, the bleaching of paper pulp, and last, but not least, the development of an apparatus for withdrawing beer from a keg, many thousands of which are today in use. It is no wonder that in 1940 the National Association of Manufacturers gave Dr. Baker the Modern Pioneer Award for Achievement in Science and Invention!

In spite of such a wide productivity in peacetime developments, Dr. Baker has been up to his ears in war activities. Under his direction, anti-desiccant compounds have been developed in the form of an ointment which is applied to the skin in order to counteract the effects of mustard gas. Azochloramid compounds have been developed and these are widely used, not only for sterilizing dishwater, but for therapeutic applications as well. Fungicides have been produced that are specific for athlete's foot and these compounds have been widely used by the United States Army. These products have been largely responsible for the remarkably low incidence of athlete's foot in the active theaters of operation. Pills for chlorinating and dechlorinating drinking water in individual canteens have been produced literally by the millions, thereby making a safe supply of drinking water available to our armed forces, and consequently cutting down on disease. Numerous other developments might well be listed as activities directly related to the war effort but time does not permit a reference to each of them. Suffice it to say that Dr. Baker and his associates have been doing yeoman service in these critical times.

I have made scarcely any reference to a series of highly significant and very interesting contributions in the field of cereal chemistry and I am assuming that we are, for the most part, familiar with these contributions. Dr. Baker has baked bread without a crust by applying heat electrolytically and has been able thereby to draw certain conclusions as to what goes on when bread dough is mixed, fermented, and baked into a loaf of bread. He has studied mixing under pressure and under vacuum and many ingenious gadgets have resulted from his intense and insatiable curiosity as to what goes on when bread is baked. Our toastmaster, Dr. Ferrari, was asked in 1942 to present a paper before the American Society of Bakery Engineers, in which outstanding recent contributions in the field of cereal chemistry were to be reported upon. Two-thirds of this paper was devoted to the contributions of Dr. Baker and his associates. Surely, in the field of milling and baking, Dr. Baker's research is the sort you can "get your teeth into."

Dr. Baker has been smart. He has shown good judgment. Early

in his career he associated himself with an organization that was young and virile and that could provide an ideal environment for invention and practical research. Associated with him these many years has been that flower that was certainly "born to blush unseen," Mr. Charles F. Wallace, himself a wizard at invention. To say which of these two profited most from association with the other would be difficult indeed.

Dr. Baker in the Laboratory

One or two characteristics of Dr. Baker, with which I was much impressed in my early association with him, are illustrated by the following anecdotes.

One day he entered the laboratory and found me weighing out a small sample of flour on the fine balance and passed the remark that he would have more confidence in the results if I started with one hundred pounds of flour, weighed on a platform scale. Subsequently, it was my privilege to listen to a debate on the subject, "What constitutes a representative sample of wheat or flour?" and it was only then that I realized the import of John Baker's offhand remark.

On another occasion, when interested in that portion of flour that is soluble in petroleum ether, he burst forth with the remark, "Let's build a giant Soxhlet extractor big enough to hold half a ton of flour—well, er, a hundred pounds, anyway." I don't recall how big the Soxhlet was but it held a lot of flour, I can assure you.

When Henry Ford moved the laboratory of Thomas Alva Edison from Menlo Park, New Jersey, to Greenfield Village, near Detroit, he had the rare good sense to move, along with the laboratory, the dump that for many years was the catchall for the inventions of the "Wizard of Menlo Park" that didn't pan out. I passed through Menlo Park twice every day during my college years and I can well remember this famous dump, overgrown with the usual varieties of weeds one finds in such a locale. I do not recall that there is such a dump near Wallace and Tiernan's factory in Belleville, New Jersey, but it seems to me I recall reading that after much legal fireworks and the passing of many laws, the Passaic River, that flows behind the plant, has finally been cleared of all contamination! For not all of the inventions and developments that have come from the fertile brain of John Baker have been outstanding successes in industry. I can well remember one—Pando—"an old friend in a new form." This was a canned liquid, resembling evaporated milk in appearance, and it comprised lard and water in the proper proportions for blending with flour, the result being a truly delicious piecrust. At one time, it was hoped that, although Mrs. Newlywed's biscuits might exhibit the texture of sole leather, she just couldn't miss when it came to baking the favorite American dessert with Pando. This same melange, when properly blended with the yolk of an egg and some vinegar, underwent a reversion of the emulsion phase and became mayonnaise dressing; but somehow the whole project died a natural death.

Dr. Baker's first recorded chemical observation was made when he was approximately six years of age. At the time, he was busily engaged in prospecting through the kitchen cupboards at his grandmother's home. Although he claimed at the time he was looking for cookies, he opened every bottle he could get his hands on and at least smelled the contents until he came to a bottle that contained household ammonia, the fumes from which knocked him completely off the stool. It was then that he made an observation on the characteristic, acrid odor of gaseous ammonia, and history records that he exclaimed, "Wow!"

During his earlier years with the Wallace and Tiernan organization, the Bakers lived in Nutley, New Jersey, and shortly after they moved there, a neighbor called on Mrs. Baker. At that time, young Betty was at the tender age of about five years and Mrs. Baker asked her to entertain the caller until she, herself, could put in an appearance.

"I suppose you go to Sunday School," said the kindly neighbor.

"No, I go to church," responded little Betty.

"What church do you go to?" asked the neighbor.

To which Betty replied, "I'm Catholic and Mother's Catholic—and Daddy's a Chemist."

Here, indeed, is an observation in which we all, I am sure, concur. Dr. Ferrari, in presenting the name of Dr. John C. Baker to Dr. Skovholt, as a candidate for this, the highest award of the American Association of Cereal Chemists, I am sure you do so re-echoing the words of his little daughter, Betty, "Daddy's a Chemist."

SELECTED BIBLIOGRAPHY OF DR. BAKER'S PUBLICATIONS AND PATENTS

Papers

†1916 Experiments upon starch as a substrate for enzyme action (*with* H. C. SHERMAN).
J. Am. Chem. Soc. **38**: 1885–1904.

†1918 The preparation of pure casein (*with* L. L. VAN SLYKE).
J. Biol. Chem. **35**: 127–136.

1918 A method for making electrometric titrations of solutions containing protein (*with* L. L. VAN SLYKE).
J. Biol. Chem. **35**: 137–145.

1918 Free lactic acid in sour milk (*with* L. L. VAN SLYKE).
J. Biol. Chem. **35**: 147–178.

†1919 Carbonic acid and carbonates in cow's milk (*with* L. L. VAN SLYKE).
J. Biol. Chem. **40**: 335–344.

†*1919 Conditions causing variation in the reaction of freshly drawn milk (*with* L. L. VAN SLYKE).
J. Biol. Chem. **40**: 345–355.

†*1919 A method for the preliminary detection of abnormal milk based on the hydrogen ion concentration (*with* L. L. VAN SLYKE).
J. Biol. Chem. **40**: 357–371.

1919 A method for determination of the keeping quality of milk (*with* L. L. VAN SLYKE).
J. Biol. Chem. **40**: 373–382.

†*1920 Reaction of milk in relation to presence of blood cells and of specific bacterial infections of the udder (*with* R. S. BREED).
J. Biol. Chem. **43**: 221–235. (Also published as New York Agr. Expt. Sta. Tech. Bull. 80 (1920).)

1920 Relation between lactic acid production and bacterial growth in the souring of milk (*with* J. D. BREW and H. J. CONN).
New York (Geneva) Agr. Expt. Sta. Bull. 74.

†1922 Flour bleaching reagents.
J. Am. Assoc. Cereal Chem. **7**: 108–111.

1925 Bleaching textiles with chlorine and its compounds.
Proc. Am. Assoc. Textile Chem. Colorists 1925, 151–153. Am. Dyestuff Reptr. **14**: 451–453.

1925 Chlorine in sewage and waste disposal.
Ind. Eng. Chem. **17**: 1059–1060.

† Papers which have been widely quoted.
* Most important contributions.

1926 Use of chlorine in the treatment of sewage.
Surveyor **69**: 241.

1926 Chlorine in sewage and waste disposal.
Can. Engr. **50**: 127–128.

1931 Ammonia-chlorine process and its application in the treatment of water (*with* F. C. SCHMELKES).
Research Publ. 305, 3–15; U. S. Pub. Health Eng. Abstracts **11** (W): 69.

1933 Measurement of color in flour and bread by means of Maxwell Discs (*with* H. K. PARKER and F. B. FREESE).
Cereal Chem. **10**: 437–446.

1935 Measurement of color in flour and bread by means of Maxwell Discs. II (*with* H. K. PARKER and F. B. FREESE).
Cereal Chem. **12**: 17–24.

1937 Mixing doughs in vacuum and in the presence of various gases (*with* M. D. MIZE).
Cereal Chem. **14**: 721–734.

1939 Relation of mechanical stirring to sponge doughs (*with* M. D. MIZE).
Cereal Chem. **16**: 294–295.

1939 Some observations regarding the flavor of bread (*with* M. D. MIZE).
Cereal Chem. **16**: 295–297.

1939 A method and apparatus for testing doughs.
Cereal Chem. **16**: 513–517.

1939 Effect of temperature on dough properties. I (*with* M. D. MIZE).
Cereal Chem. **16**: 517–533.

1939 Effect of temperature on dough properties. II (*with* M. D. MIZE).
Cereal Chem. **16**: 682–695.

1939 The permeability of bread by air.
Cereal Chem. **16**: 730–733.

1941 The origin of the gas cell in bread dough (*with* M. D. MIZE).
Cereal Chem. **18**: 19–34.

1941 The structure of the gas cell in bread dough.
Cereal Chem. **18**: 34–41.

1942 The relation of fats to texture, crumb, and volume of bread (*with* M. D. MIZE).
Cereal Chem. **19**: 84–94.

1942 The action of an oxidizing agent in bread dough made from patent flours (*with* H. K. PARKER and M. D. MIZE).
Cereal Chem. **19**: 334–346.

1943 The pentosans of wheat flour (*with* H. K. PARKER and M. D. MIZE).
Cereal Chem. **20**: 267–280.

1943 Measurement and significance of gluten quality (*with* M. D. MIZE and H. K. PARKER).
Cereal Chem. **20**: 506–516.

1944 The action of oxidizing agents on sulfhydryl compounds in dough (*with* H. K. PARKER and M. D. MIZE).
Cereal Chem. **21**: 97–107.

Patents

U. S. 1,354,683	Manufacture of milk fat. E. B. Phelps, A. E. Stevenson, and J. C. Baker (Assigned to A. W. Johnston)	October 5, 1920
†**U. S. 1,367,530*	Bleaching and maturing flour and other cereal products. (Agene process) J. C. Baker	February 8, 1921
U. S. 1,378,644	Process of sterilizing with nitrogen trichloride. (NCl_3 sterilization) J. C. Baker	May 17, 1921
†*U. S. 1,403,993*	Manufacturing hypochlorite solutions. (Industrial bleach panel) C. F. Wallace and J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	January 17, 1922
U. S. 1,404,922	Bleaching and maturing flour. J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	January 31, 1922
†*U. S. 1,413,092*	Milk fat. J. C. Baker	April 18, 1922
†*U. S. 1,413,153*	Method of sterilizing water and sewage. (Limestone tower) J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	April 18, 1922
†*U. S. 1,413,154*	Bleaching paper and fabrics. (Neutral hypochlorite bleach) J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	April 18, 1922
U. S. 1,468,693	Bleaching paper pulp. J. C. Baker	September 25, 1923
†**U. S. 1,510,132*	Process of producing nitrogen trichloride. J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	September 30, 1924
U. S. 1,553,294	Shortening compound. J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	September 8, 1925

† Patents in use.
* Important patents.

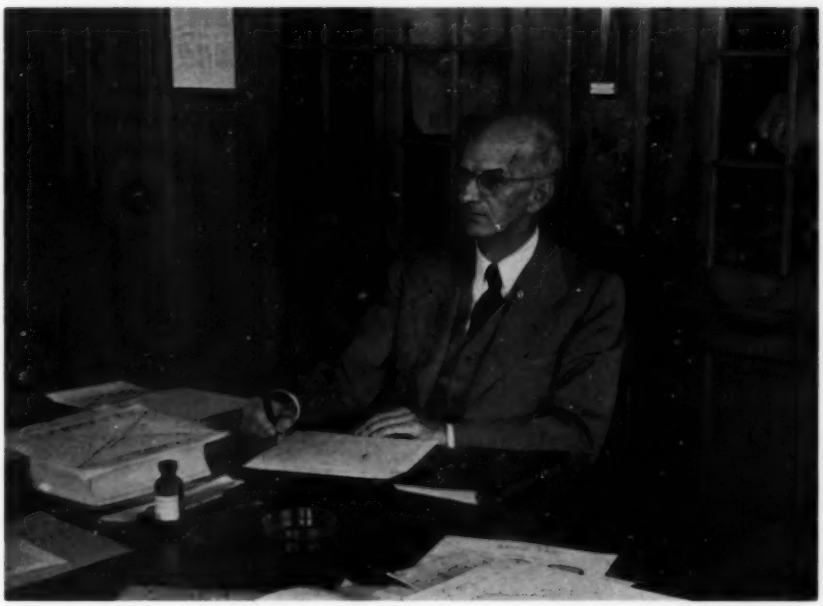
U. S. 1,678,625	Apparatus for the therapeutic application of chlorine. (Chlorine inhalator) C. F. Wallace & J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	July 24, 1928
†**U. S. 1,745,141*	Prevention of formation of scums and films. (Intermittent desliming) (Condenser water treatment) J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	January 28, 1930
†*U. S. 1,794,134*	Colorimeter. (Residual chlorine comparator) J. C. Baker and C. F. Wallace (Assigned to Wallace & Tiernan Products, Inc.)	February 24, 1931
U. S. 1,834,759	Rubber printing plate. J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	December 1, 1931
U. S. 1,850,057	Method of making dichloramine. J. C. Baker and F. C. Schmelkes (Assigned to Wallace & Tiernan Products, Inc.)	March 15, 1932
U. S. 1,850,058	Method of making monochloramine. J. C. Baker and F. C. Schmelkes (Assigned to Wallace & Tiernan Products, Inc.)	March 15, 1932
†**U. S. 1,850,975*	Method of making hypochlorite solutions. (Laundry equipment) J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	March 22, 1932
U. S. 1,866,524	Improving taste and odor of water. J. C. Baker and F. C. Schmelkes (Assigned to Wallace & Tiernan Products, Inc.)	July 12, 1932
†**U. S. 1,923,044*	Apparatus for use of chlorine in treating water. (Silica gel protector) J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	August 15, 1933

†*U. S. 1,937,865*	Use of fused silver chloride for joining glass to metals such as in joints of chlorine supply apparatus. (Glass-to-metal joint) J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	December 5, 1934
U. S. 1,943,650	Method of sterilizing water with dichloramine. J. C. Baker and F. C. Schmelkes (Assigned to Wallace & Tiernan Products, Inc.)	January 16, 1934
†**U. S. 1,960,615*	Measuring and controlling apparatus. (Residual chlorinator) J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	May 29, 1934
†**U. S. 1,967,074*	Process of treating fresh fruit. (Decco) J. C. Baker	July 17, 1934
†*U. S. 1,995,914*	Method and apparatus for treating water. (Close Injector–Impulse Diffuser) J. C. Baker and G. D. Peet (Assigned to Wallace & Tiernan Company, Inc.)	March 26, 1935
U. S. 2,011,171	Electrolyzing apparatus suitable for chlorine production. (Electrolytic cell) J. C. Baker (Assigned to Wallace & Tiernan Company, Inc.)	August 13, 1935
†**U. S. 2,165,605*	Beer drawing apparatus. J. C. Baker (Assigned to Standard Brands, Inc.)	July 11, 1939
U. S. 2,233,372	Method for testing dough. J. C. Baker (Assigned to Wallace & Tiernan Products, Inc.)	February 25, 1941
†**U. S. 2,326,278*	Making of bread. J. C. Baker (Assigned to Standard Brands, Inc.)	August 10, 1943

†**Canadian 212,259*	Process of bleaching and maturing flour. J. C. Baker	June 28, 1921
†**Canadian 263,831*	Nitrogen trichloride. J. C. Baker	August 24, 1926
†*Canadian 287,408*	Method of making hypochlorite. (Laundry equipment) J. C. Baker	February 26, 1929
†*Canadian 340,630*	Silica gel protector.	April 3, 1934
Spanish 134,906	Process for treating fruit. J. C. Baker	July 13, 1934
†**British 232,607*	Nitrogen trichloride in bleaching flour. Wallace & Tiernan Company and J. C. Baker	April 21, 1924
†**British 345,637*	Condenser water treatment. J. C. Baker	June 18, 1931
†*British 398,124*	Means for preventing water containing chlorine from reaching apparatus for controlling the supply of chlorine for the treatment of water. (Silica gel protector) J. C. Baker	September 7, 1933
†*German 590,805*	Silica gel protector. J. C. Baker	January 21, 1933
†*German 378,421*	Bleaching and maturing flour and other cereal products. (Agene process) J. C. Baker	December 21, 1920

C. G. Ferrari: Yes, indeed, we all agree that "Daddy is a chemist" and an exceptional one, else we would not be gathered here tonight to do him honor.

It adds much to this occasion to have with us this evening the president of our national Association, Dr. Oscar Skovholt. It will be his privilege to confer the Osborne medal. Dr. Skovholt, as chairman of the Osborne Medal Award Committee, I am happy to present for this honor Dr. John C. Baker.



DR. BAKER IN HIS OFFICE

PRESENTATION OF THE THOMAS BURR OSBORNE MEDAL

OSCAR SKOVHOLT, *President, American Association of Cereal Chemists*

On this occasion it seems appropriate to review briefly the objectives of the American Association of Cereal Chemists. It is likely that few of those present are familiar with Article II of the constitution which defines the "purposes of the Association." This article reads in part as follows: The purposes of this Association are (1) the encouragement and advancement of scientific and technical research in cereals and their products, particularly in milling and baking, but including also other industries in which cereals and their products are utilized, (2) the study of analytical methods used in cereal chemistry and the development and adoption of uniform (or standard) methods of examination and analysis, (3) the promotion of the spirit of scientific cooperation among all workers in the field of cereal knowledge, (4) the maintenance of high professional standards in the Association as conditions of membership, and (5) to encourage a more general recognition of the chemist and biologist as essential factors in the development of the cereal industries.

The Osborne Medal Award spotlights the work of an outstanding individual, thus directly helping to attain one of the purposes of the Association, namely, that of "encouraging a more general recognition of the chemist and biologist." Such a recognition of one scientist brings a small amount of reflected glory to all who have contributed to the advancement of cereal chemistry. The attainment of the other purposes of the Association is aided by the stimulus to all members obtained through these Osborne Medal Award occasions and by the prestige that is gained by the sponsoring body.

These are the advantages to the Association and its individual members from the medal award. We do not think of these occasions in terms of material benefits, however, but obtain genuine pleasure from the opportunity thus afforded to honor one of our own.

On the face of this medal is the inscription "For Distinguished Contributions in Cereal Chemistry." Contributions can take many forms. Tonight's medal recipient has been a leader in directly accumulating much valuable information. But these collected facts

may be overshadowed in importance by the stimulus given to research in many organizations from the new concepts that he has frequently thrown out as sort of a challenge to all cereal chemists. Most of us will remember with pleasure and profit the many public and private discussions brought forth by the work of Dr. Baker and his associates. All of us who have been active in Association affairs know that every acquaintance is his friend. Those of us in industry are particularly pleased that this important recognition has come to one engaged in the same type of endeavor.

Dr. Baker, will you please arise. It is a real pleasure to award this medal to one who has helped so much in unraveling some of the mysteries surrounding flour and dough behavior, who has greatly stimulated thinking and research, and who, above all, is so genuinely loved by every member of the Association. It is a privilege to act for the Association in presenting to you, Dr. John C. Baker, this Osborne medal.

ACCEPTANCE OF THE OSBORNE MEDAL BY DR. BAKER

President Skovholt, Chairman Ferrari, Dr. Dunn, Mr. Karrh, members of the American Association of Cereal Chemists, and my many dear friends:

It is with the deepest joy and greatest appreciation that I accept the Thomas Burr Osborne Medal awarded in recognition of my researches in cereal chemistry. Though only my name appears on its face, the work which you have thus honored was the result of teamwork, a three-horse team or possibly two horses and a mule, though I am sure no one of us will acknowledge being the latter. Dr. Hugh K. Parker and M. D. Mize are the other members of that team. Two finer associates one could not find. Better personal and friendly working relationship could not exist. We three have worked, pulled, argued, experimented, planned, and written together. Much of the knowledge obtained has come through M. D.'s skilled fingers, or from Hugh's patient, careful analyses. Their names might well have been engraved deep in the golden surface of this medal beside mine. Any joy it gives is also theirs and this acknowledgment is ours together.

There are others who share this award. Mr. Wallace and Mr. Tiernan, who have so happily given this reception here this evening, are my bosses but, more than that, they are my close friends and associates who have made this teamwork possible. We have always had their encouragement and often their direct help, as well as that of the staff of their company. How could one fail when working for such men? They, too, share in this honor.

Many others on the rolls of the company have been of help to me in acquiring the knowledge and experience that led us to attack cereal problems. In particular, I must acknowledge the service of William Farrell, who was the first to bring to me practical information on experimental baking. It was he who made the first baking of Agene-treated flour. Then, too, John Hasselman, with his wealth of experience, taught me much about this art. Also, Bert D. Ingels was of particular help to me because he would always listen and always encourage. Of what great value is a patient ear into which one can pour out his stories of searching truth!

Though I have never had any other ambition than to delve into science, my college education in furtherance of this desire was meager and inadequate. While at school I met the girl who later became my wife. She has backed me at every move. After teaching elementary sciences in North Dakota and later in New Mexico, the teaching profession lost its interest for me. I had been trying experiments in cracking oils and had made a survey of food adulteration in New Mexico and felt such lack of basic knowledge in this work that we (I was married by then) took our small savings and went to Columbia University. There my education began. I want to mention a few men from that school who helped me. First was the late Alexander Smith, the greatest chemistry teacher I have known. Among others were Hal T. Beans, J. M. Nelson, Floyd Metzger (whose assistant I was), and last, but not least, Henry C. Sherman, under whom I majored. At that time, 1916, I attended a lecture by two visitors. They talked jointly of their work in determining the biological value of the amino acids. (How modern that sounds!) There, they disclosed the methods of animal feeding that later led to the discovery of vitamins and their assay, as well as to the evaluation of foods. One of those men was Thomas Burr Osborne, whose picture is here embossed, and for whom this medal was created. He was not only the father of protein chemistry in this country, but truly started our modern science of nutrition on its way. No more worthy name could have been so honored.

Among the many friends I made while at Columbia, one greatly influenced my life. The late Dr. Glenn E. Cullen and I worked and studied together. He induced me to work with him at Rockefeller Institute for a while, and there introduced me to the Junior Van Slyke and to his father, Dr. L. L. Van Slyke. This led me to my first job, which was in dairy chemistry at Geneva, New York. There I spent three fruitful years in independent research without assistance or guidance and learned the full joy of discovery and new knowledge.

The shift from there to my present work I owe to Professor Sherman. My interest in cereal chemistry I owe to Mr. Tiernan. His tale of patent difficulties in selling chlorine machines to flour mills led me to experiment in that field with the prompt discovery of the action of nitrogen trichloride. I approached the Hecker Jones Jewell Milling Company in New York and there met Chauncey Foster, who guided my first steps in cereal chemistry. It was he who first taught Harry Snyder about milling, and Foster received, in exchange, training

in chemistry. So, my first contact with our science of cereal chemistry carries me back to its early history in this country.

My first cereal publication in 1922 was the result of that training. The paper dealt with flour bleaching. Many years elapsed before I obtained the experience, confidence, or time necessary to engage in further cereal research.

Active studies were started again after a trip to England in 1935 and a visit to Kent-Jones' laboratory. There I saw in use every device then known, including substantially all of those now in use. After Dr. Amos (Kent-Jones' associate) had taken me through their extensive laboratories, I asked him on what they based their judgment of flours. He introduced me to their baker and said, "On this man's judgment." I came home determined to devise a machine that would do the work. The result was not the machine but this medal. Like a bird dog hunting for quail but which starts a rabbit instead, I couldn't resist. The old research desires came back, so with the whole pack we chased those rabbits hoping we might also find a quail.

Dr. Ferrari has asked me to tell you something of our work. From this I will deviate and try to give you a brief picture of dough and bread as I see it, based on our researches plus the accumulated knowledge of cereal chemistry and some imagination.

BREAD AS I SEE IT

JOHN C. BAKER

Bread dough is made by mixing together flour, water, salt, and yeast. Other materials such as sugar, fats, milk, or conditioners may be added. The resulting familiar plastic and elastic dough mass is constructed of not less than six physical entities or phases, which make up its volume and control its properties. Five of these phases, i.e., starch, an aqueous solution, gluten, fats, and yeast, comprise the solid and liquid portions of the dough. Gas forms a sixth phase, which is present in widely varying amounts. Starch occupies about one-half the volume of gas-free dough. The remaining portion lying between the starch granules is occupied by a semiliquid mixture containing all the other phases. About two-thirds of this liquid mixture is free aqueous solution. The remainder of the liquid mixture consists of hydrated gluten. Flour fat is largely located in the gluten. Yeast is distributed in the aqueous solution. These dough phases can be partially separated from each other in a supercentrifuge.

With this physical picture of dough structure in mind we shall consider some of the properties of the separate phases and their relation to the whole.

Wheat starch is notable among cereals because of its wide variation in granule size. This gradation permits closer starch packing and hence requires more separation of granules to accommodate the other dough materials, resulting in greater mobility than would be obtained from uniform-sized granules.

Wheat starch on its surface carries pentosan, protein, and lecithin. These surface materials doubtless affect dough mobility and possibly provide a semipermeable layer that permits the quick passage of water to the starch interior but which resists the passage of dissolved materials. Also, the surface coatings of wheat starch enable it to resist diastatic attack. Thus, in breadmaking only the damaged starch grains are quickly changed to sugar, and prolonged action is required to convert appreciable amounts of the remainder. Proteases, pentosanases, or lipases, by attacking the surface coatings, may thus aid the action of diastase.

A liquid phase can be separated from dough by supercentrifuging. This yields a highly viscous solution, which may upon oxidation coagulate to a gel and which can be whipped like white of egg to a stiff foam. This liquid comprised most of the water added to make dough and carries in solution pentosans, proteins, carbohydrates, salts, enzymes, and gases, which are all quickly dissolved when the dough is first formed. The soluble pentosans are largely the source of high liquor viscosity and also its gelling reaction. The soluble nitrogen compounds, which are mostly albumins and globulins of low molecular weight, provide the whipping qualities of the liquor. These soluble proteins may contain sulfur compounds in the reduced state which have a marked action on the gas-retaining qualities of dough.

The gluten phase, which also separates from dough in the supercentrifuge, is unique among proteins because it tends to cohere and form an elastic substance upon rubbing or stirring. This cohering property is affected by many substances. It is hastened by some salts, by oxidizing agents, and by either reduced or oxidized glutathione type compounds. Gluten coherence is retarded by the liquid phase of dough, also by many reducing compounds, alcohol, urea, acids, alkalies, and certain salts, such as salycilates.

The action of salts which promote gluten formation is of particular interest. In their complete absence gluten coherence does not occur. Gluten obtained from minimum salt concentrations is easily dispersed in distilled water forming a solution which is permanent and not separated by supercentrifuging. The addition of salts to such a solution quickly precipitates the gluten. Upon working, this sticky mass forms the usual rubbery substance. If saturation with sodium chloride is used for precipitation along with violent agitation, the gluten forms as a fine nonsticky powder which does not cohere under pressure or rubbing. If this powder is leached with distilled water, it swells and takes on the appearance and character of alumina gel. If, before the salt is all leached out, such a tender gel is rubbed, elastic gluten again forms.

These experiments show that salt is required to activate cohesion of the gluten particles, and rubbing is required to bring them together to form gluten. The gluten in dough at the start of the mixing period is possibly similar in form to that in the "unrubbed" gel. The gluten that develops in the gel upon rubbing is probably similar to the gluten network that forms in dough in the liquor phase around the starch during dough mixing.

Gluten doubtless exists in wheat as submicroscopic micelles of near globular form, embedded in the solubles. Upon wetting, these solubles immediately dissolve, permitting the gluten to hydrate, soften, and become sticky. Upon mixing, the protein micelles are forced into contact and cohere, after which the stretching action of mixing pulls these gluten aggregates into a more elongated shape. This strain unfolds or increases the surface of the gluten particles, thus increasing its cohesive capacity to varying degrees. Possibly herein may be found the cause of gluten quality.

The cohesive property of gluten may be largely mechanical in nature. The working of gluten produces effects similar to those observed in felting of hair, or as obtained by working a mass of minute coiled springs in the hand. Chemical forces are also involved, as shown by the need of salt and the effects of oxidizing and reducing substances. The exact nature of the gluten mechanism is one of the many unsolved problems of cereal chemistry.

A gas phase is always present in dough. The gas may originate from voids in the flour, from air occluded during mixing, or from fermentation. The yeast organism does not initiate the gas phase. Its generated carbon dioxide is dissolved in the dough liquid and must diffuse into existing voids. Manipulation of dough may add, subdivide, or eliminate gases. Gases are occluded at an increasing rate during the development period of dough mixing. The rate of gas occlusion becomes fastest as the maximum resistance of the dough to mixing is reached. Thereafter gases are occluded more slowly. Gas dispersion during mixing of dough thus accompanies the cohesion of the gluten particles that produces the increased resistance to mixing. This suggests that gas particles are trapped in the dough by the gluten mesh as it establishes its continuity in the liquid phase. Small gas particles or bubble nuclei are thus formed in and surrounded by connecting gluten. Such bubbles are able to retain the fermentation gas that diffuses within and causes their expansion. When doughs are undermixed the occluded gas bubbles are not able to expand without rapid leakage of gas and coalescence. Such bubbles as do expand rise through the dough because there is no connecting gluten structure to resist their movement. Additional work of punching or moulding is required on such undermixed dough to develop the gluten contacts and produce cohesive membranes around the gas bubbles.

In an expanding bubble the surrounding gluten network is under continuous and increasing strain which will cause it to either flow or

break to relieve the tension. The flow of the glutinous bubble wall is promoted by substances in flour which weaken the cohesion of the gluten particles. The reduced sulfur compounds of flour produce this effect. Oxidation or prolonged fermentation may decrease the action of these sulfhydryl compounds, resulting in more persistent cohesion.

The hundred-fold expansion of the bubble in breadmaking is accomplished by flow of the glutinous cell wall rather than by its elastic stretch. The flow of gluten can be explained as a continual separation of cohered particles as other cohesions are established, and suggests that in well-developed dough the gluten possesses large reserves of cohesive force that come into use as others are released to permit bubble expansion without breaking. The gluten's quality is characterized by its ability to flow without breaking and without completely releasing elastic strain.

When dough is proofed to the desired volume and placed in the oven to be baked, heat causes increased pressure, producing sudden strain on the cell structure that must not fail until the starch by swelling and hydrating stiffens the dough. Thereafter the cells do not lose their identity, but they must either rupture or leak to release the pressure. The leakage of gas from cells in dough during baking is related to the fat phase of dough which is semiliquid at fermentation temperatures. When cell walls become extended and thin, these fat particles, as they are warmed and become freely liquid, provide the escape channels for gas without breakage of the walls. The entire dough thus becomes porous. Hence, from nonshortened doughs, where the flour fat is more nearly a liquid, bread of fine cell structure but of moderate volume may be obtained. By adding fats of higher melting point or increased viscosity, the holes in the cell walls can be closed. This plugging of the gas escape mechanism causes the cells to expand further but they soon explode because of the very fast release of carbon dioxide and alcohol gases by heat. Thus, bread made with shortening has a cell structure that is broken and torn. The bread is more tender because of these breaks and dries out faster because air permeates more freely.

To obtain optimum bread there is required (1) a freely fermenting dough that has been manipulated to contain the maximum number of gas cells, (2) one that has been developed so the gluten will retain continuity without breaking, and (3) one that has had shortening added to plug the leaks. When such a dough is properly proofed and baked, its maximum breadmaking capacity is obtained. The

resulting large loaf has a bold shape, thin crust with an appealing color, finely shredded unbroken side walls, and an interior crumb that has uniform fine pores of elongated shape and shiny glutinous walls. Though such a loaf may usually not be obtained or desired in commercial practice, the knowledge by which it can be achieved is necessary to maintain a desired standard in the milling and baking industries.

What has been presented to you this evening represents, as concisely as I can give it to you, a resume of what is known about certain phenomena taking place in the dough mass, together with some of my personal views and interpretations, based in part on some as yet unpublished experimental data. Much still remains to be done before a complete picture can be drawn, but I cannot help but feel that progress is being made towards a solution of this interesting, complex, and important problem.

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